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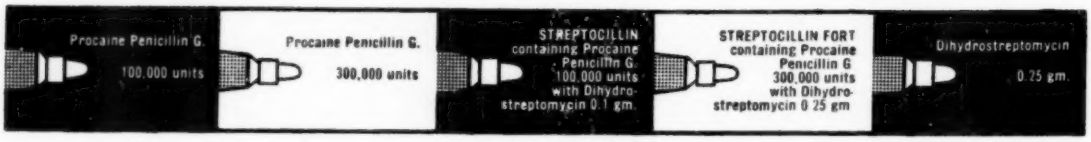

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The Ecology of the Liver Fluke *(Fasciola hepatica)*

BY

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SUMMARY—The usual diagram of the life cycle of F. hepatica *shows successive phases of development of the parasite as they would ideally happen and without any notice of mishaps on the way. There are, in fact, no fewer than six phases:* (1) *transference from the host to the ground;* (2) *development within the egg;* (3) *transference from the egg to the snail;* (4) *development within the snail;* (5) *transference from the snail to the final host via the herbage;* (6) *development within the final host. Each phase has its hazards, and in each there is the occurrence of a lesser or greater loss of numbers.*

Observations carried out at Weybridge have shown that the most important factors influencing the success in the development of this parasite concerned moisture and temperature. The interaction between these two factors and the parasite is analysed and discussed. It provides a basis for understanding the epidemiology of fascioliasis.

Introduction

OVER thirty years ago carbon tetrachloride was introduced for the control of fascioliasis in sheep and it would be fair to say that it still remains the drug of choice. The fact that there has been little or no indication of the appearance of a drug markedly more effective than carbon tetrachloride is probably the reason why nearly all accounts of research on fascioliasis have concentrated on the life cycle of the fluke, originally described by Thomas in 1883, and on the life history of the vector snail. Here again, in spite of considerable effort in various parts of the world, there has been no discovery that would suggest an easy and economical solution to the problem of the disease in the field.

Although the life cycle of *F. hepatica* presents many aspects for study, certain stages of the life cycle are easier to observe and measure than are other stages, and, as might be expected, there has been a tendency to study these in more detail. Inherent in this approach is a danger that stages which are relatively easy to study may assume greater significance in the life cycle than would be the case if all could be studied with equal ease. The intermediate snail host *Lymnaea trunculata*, for just such a reason, has been the subject of much study, with the result that it has assumed great prominence in the life history of the parasite. For instance, the serious outbreaks of acute fascioliasis which were experienced on many farms during the winter of 1958 were attributed by many to the presence of large numbers of snails which were the result of favourable conditions for their breeding during the previous summer (*Vet. Rec.*, **71**, 57). Although the presence of the snail is obviously essential if the life cycle is to occur, such a simple explanation as this is not necessarily correct.

It seems appropriate, therefore, to review the stages in the life cycle of the parasite from an aspect different from that to which one is accustomed; equal consideration may then be given to all stages in the life cycle, as a result of which it may be possible to evaluate their relative importance.

The Life Cycle

The usual diagram of the life cycle of *F. hepatica* shows each stage of the parasite developing into the next stage either outside or inside the intermediate and final hosts. A moment's reflection shows that this idealised cycle does not necessarily occur; in order to complete the life cycle, each stage not only has to develop in a particular environment, but, having done so, it has to pass from that environment into the next, where the succeeding stage is to develop. As reported by Michel and Ollerenshaw (in press), it may be of value to regard the life cycle as an alternation of developmental and transport phases. Of great importance is the fact that in each developmental and transport phase a varying degree of mortality occurs. This is represented in Fig. 1 by the large arrows which diverge from the circle. Representation in this way

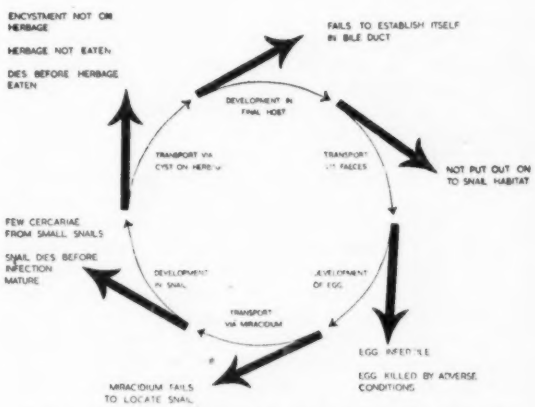
ENCYSTMENT NOT ON HERBAGE
HERBAGE NOT EATEN
DIES BEFORE HERBAGE EATEN
FAILS TO ESTABLISH ITSELF IN BILE DUCT
DEVELOPMENT IN FINAL HOST
TRANSPORT VIA CYST ON HERBAGE
TRANSPORT VIA FAECES
NOT PUT OUT ON TO SNAIL HABITAT
FEW CERCARIAE FROM SMALL SNAILS
SNAIL DIES BEFORE INFECTION MATURE
DEVELOPMENT IN SNAIL
DEVELOPMENT OF EGG
TRANSPORT VIA MIRACIDIUM
EGG INFECTILE
EGG KILLED BY ADVERSE CONDITIONS
MIRACIDIUM FAILS TO LOCATE SNAIL

FIG. 1.—Diagram of the life cycle of *Fasciola hepatica*.

stresses the fact that mortality is normally high and counterbalances the reproductive potential of the parasite. Mortality may vary from time to time within a phase, and also from one phase to another. These variations in mortality will be responsible not only for the absence or presence of the disease in an area, but also for the varying incidence of the disease when it does occur. It is to be expected that the variations of greatest magnitude in time will exert the greatest effect on the parasite.

Study of the parasite in each of these phases, particularly in relation to the environment, is likely to

reveal those factors which induce mortality, and those which facilitate successful development or transport to the next phase. Such study will help in understanding the epidemiology of the disease, and ultimately may be of value in its control.

Developmental Phase in the Final Host

Before the life cycle can occur in any area, the presence of animals infected with the parasite is a necessary prerequisite to subsequent infection. As a disease in this country, fascioliasis is most obvious in sheep, yet cattle and rabbits are important final hosts. Resistance to the parasite by the final host, such as is seen in many nematode infections, appears to be of little importance in reducing the number of flukes or their output of eggs. The only factors likely to influence the spread of infection in this respect are the distribution, management and dosing of infected animals.

Examination of faecal samples from cast ewes and cattle on a hill farm in Kincardineshire in 1954 showed that almost 80 per cent. of the cast ewes and 60 per cent. of the cattle were lightly infected with the parasite. These findings were on a farm where fascioliasis as a disease was not considered a problem, and where dosing was little practised. Examination of random samples of faeces collected on ten farms in Anglesey during the summer of 1958 revealed an 85 per cent. infection in ewes which had been dosed as recommended during the previous autumn and winter. It is well known that little routine dosing of bovine animals is practised in this country, yet reports from the abattoir in Exeter for 1956 show an average of 58 per cent. of the cattle slaughtered to be infected. Similary, the O.E.E.C report on "Control of Losses in Young Farm Animals, 1957," states that 95 per cent. of all cattle in Ireland are infected. In view of these facts, and bearing in mind the length of time for which the fluke can remain in the final host, the constant interchange of stock from farm to farm, the importation of many Irish cattle into this country and the difficulty, even with the help of myxomatosis, in eradicating the rabbit it seems certain that on the vast majority of farms in Britain some at least of the farm stock and rabbits will harbour the parasite. It can be concluded, therefore, that absence of mammalian infection is unlikely to be an important limiting factor in the spread of the disease, and that at least a few fluke eggs will be present in dung dropped on to most pastures throughout the year in Britain.

Transport Phase of the Fluke Egg

This phase is concerned with the passage of the parasite from the final host to a free living existence. Such is the nature of the succeeding transport phase (i.e., the miracidium) with its restricted powers of movement, that continuation of the life cycle is probable only where the fluke egg is deposited into a snail habitat. Whether dung is dropped into a snail habitat or not is largely a matter of chance; what is more certain is that if a snail habitat is present in the grazing area, some faeces are likely to be dropped in it. In general, the distribution of snail habitats is very localised so that the vast majority of fluke eggs which pass out of infected animals have, from the very beginning, little chance of completing the life cycle, even in those years when favourable conditions cause great expansion of snail habitats.

Description of the snail habitat will be given later; meanwhile, consideration must be given to those eggs which are deposited in snail habitats.

Developmental Phase of the Fluke Egg

The importance of temperature in the development of the fluke egg was first observed by Thomas (1883), and Ross and McKay (1929) noted that the critical temperature below which fluke eggs will not develop is 10°C. Apart from these observations, little has been reported of the conditions necessary for the development and subsequent hatching of the fluke egg in the field. I am indebted to my colleague, Mrs. Rowcliffe, for much of the following information. The results of this work show that in temperatures existing in Britain, development of the egg to the hatching stage will take not less than three and often four weeks, and that from about the end of October to the beginning of May low temperatures inhibit development.

Also of great importance are the facts that the fluke egg will not develop to the hatching stage unless it becomes separated from identifiable faecal material, and that the egg is in no way resistant to desiccation. Initially, the moist faeces cushion the effect of desiccation and viability of the egg persists for a considerable time in moist faeces. Later, however, moisture conditions in the faeces become dependent on those in the external environment and desiccation may occur within the faeces. Moreover, in dry weather, "caking" of the faeces certainly retards any subsequent disintegration which has to occur if the egg is to be liberated from faecal material. Such liberation will be accelerated if the faeces are diarrhoeic and deposited directly into water. Thereafter, survival of the egg will depend on moisture conditions in the surface layer of soil.

Observations show that the egg will develop in the surface layer of soil without the presence of free water on the soil surface, provided the soil is saturated with water. The critical level of soil moisture necessary to prevent desiccation is therefore the maintenance of field capacity. The existence of these conditions in the soil depends on several factors, the most important being the obvious one of climate. Bearing in mind the varying climate of this country, together with the specialised requirements of the fluke egg, it is to be expected that great variations in the numbers of eggs hatching will occur from time to time.

Transport of the Miracidium to the Snail

This phase is undoubtedly the most difficult to study in the field and is perhaps one of the most critical. It is well known that the miracidium has a short active life of only a few hours, during which time contact and penetration of the intermediate host must occur if the life cycle is to continue. According to Neuhaus (1953), a positive chemotactic reaction by the miracidium to *L. trunculata* occurs up to a distance of 15 cm. Beyond this distance contact between the miracidium and the snail must depend on chance. It is to be expected, therefore, that the greater the density of snails,

the greater the chance of a miracidium successfully locating an individual snail. The important factor about which nothing is known is the wastage of miracidia which may or may not occur when the density of snails is low. Information regarding the minimum density of snails necessary to prevent such wastage of miracidia would be a most valuable addition to our knowledge of the life cycle.

Developmental Phase in the Snail

Since the presence of the snail is indispensable to the life cycle of the parasite, an appreciation of the ecology of the snail will be of value before considering the developmental phase of the parasite within the snail. The response of the snail to moisture forms the basis of this appreciation.

The presence of adequate moisture is essential if the snail is to become active, grow and reproduce; its absence induces the snail to æstivate and in this state the snail is inactive—it cannot grow nor reproduce. In order to maintain snail activity, it is necessary that the soil should be at field capacity, i.e., retaining as much water as is possible against the force of gravity. Ideally, there should also be a little free water on the soil surface. Soil attains field capacity over much of this country in winter, but snail habitats are likely to develop only in those areas where field capacity, together with a little free water on the surface of the soil, occurs in late spring, summer and early autumn—that period of the year when growth of the snail is more rapid and when breeding normally occurs.

In permanent water, *L. trunculata* is replaced by other species of snails, whilst on better drained areas the snail has to æstivate for extensive periods. Very prolonged periods of æstivation are likely to result eventually in the disappearance of the snail. It will be apparent, therefore, that the snail is restricted to areas where moisture conditions are marginal between those found in permanent water and those on well-drained land.

Even in its own recognised habitat, the snail may be subjected to prolonged periods of drought. In contrast to the fluke egg, the snail is extremely tolerant to such adverse conditions, and decreases in populations do not follow immediately after the onset of drought conditions. Indeed, snails have been recovered from habitats up to eighteen months after they have been drained.

A return of favourable conditions to the habitat stimulates the snail to activity and reproduction ultimately follows. The snail, however, may have to grow before it can lay eggs, which in turn need time to hatch before an increase in population can occur, and although increases of from less than 20 per square metre to more than 900 per square metre have been observed to occur in three months under natural conditions, all the daughter snails were less than 3 mm. long at the end of this time. (The significance of the size of the snail in relation to the development of the parasite is discussed later.)

Although moisture is so important in the life cycle of the snail, there are extensive areas of hill land in Britain which maintain ideal moisture conditions, but are unable to support a population of *L. trunculata*. These conditions occur on land which is continuously waterlogged and, as a result, gives rise to the formation of peat and leached soils which, because of their acidity and lack of mineral salts, preclude the presence of the snail. In these areas the distribution of the snail is restricted to flushes around springs on the hillsides and to certain badly drained valley bottom. On lowland farms the snail is commonly associated with badly drained rushy areas, shallow drainage ditches, around broken drains, seepages, wet gateways and similar wet areas.

In general, the frequency and distribution of snail habitats in any area depends on a number of factors. The topography of the land, the presence or absence of drainage systems, together with the soil type and the underlying rock formation all play an important though constant role in the formation of habitats. Even where these factors are favourable to the occurrence of snail habitats, their actual formation will ultimately depend on the rainfall in the area.

The life cycle of the snail in this country is best considered as having a basic rhythm. Parent snails commence laying eggs in spring and continue to do so throughout summer, gradually dying off during this period and usually disappearing by October. The daughter generation hatches out and grows throughout summer, more slowly during winter, and then forms the parent generation of the following year. This basic rhythm is, of course, entirely dependent on the occurrence of favourable moisture conditions during the spring, summer and early autumn. If wet weather does not occur until August, then breeding will be delayed until that time. In some years reproduction may occur in spring and again in late summer, interrupted by dry weather in July. In the event of favourable moisture conditions being maintained throughout spring and summer, a great increase, both in the number of snails and in their area of colonisation, may occur and a second generation of snails may be produced in autumn. Where an increase in the area of colonisation is not possible, exceedingly dense populations of snails may develop, an average density of just over 3,300 snails per sq. m. being observed on one occasion.

Studies on snail populations have been maintained on a number of habitats in both Wales and Scotland since 1952, and although some of these habitats have been repeatedly treated with molluscicides, no instances of the complete disappearance of the snail have been recorded unless drainage also has occurred. During the period of observation, although snail populations and their areas of colonisation have fluctuated greatly, with large populations developing in wet summers and small populations occurring in dry summers, some snails were always to be found in habitats left in their natural state. There was evidence also that in most habitats at least a little breeding occurred each year, although its location within the area of possible habitat varied according to the suitability of moisture conditions.

With this background knowledge regarding the life cycle of the vector, it is now possible to consider the relationship between parasite and snail. Although moisture conditions necessary for the hatching of the fluke egg are closely similar to those necessary for promoting activity, growth and reproduction of the snail, it has been shown that variations in the num-

bers of fluke eggs hatching are likely to be greater and more rapid than the variations in the number of snails. It is possible, therefore, for numbers of fluke eggs to hatch before there has been any great increase in the snail population. Although some snails are to be found in most habitats, it has been shown that their density may be very low, and under these circumstances it is important to determine whether the scarcity of snails may limit the development of the parasite population to a level below that necessary to produce acute outbreaks of the disease.

The evidence, as yet incomplete, suggests that herbage infestations of a level dangerous to grazing stock can, in fact, arise from snail populations of low density. Outbreaks of the disease have occurred with populations as low as 50 per sq. m. Furthermore, in any population of snails, only a small percentage harbours the parasite, usually less than 2 per cent. up to 20 per cent.—occasionally higher when conditions are favourable for the hatching of the fluke egg. In our experience the density of infected snails necessary to produce losses from the acute form of the disease may be less than 5 per sq. m. In comparison with the numbers of snails that can sometimes occur, these figures are extremely low.

Another significant factor in assessing the importance of snail numbers is the fact that the size of the snail (which reflects the state of nutrition of the snail) has an important bearing on the development of the parasite (see Kendall, 1949a). This is well demonstrated in Fig. 2, which shows the results of measuring and dissecting over 6,800 snails, of which over 1,000 were infected when collected in Anglesey during the summer and autumn of 1958. It shows, quite clearly, that in snails carrying a mature infection, the largest snails may harbour almost ten times the number of rediae which a small snail might bear. Furthermore, individual rediae in large snails are invariably much larger than those in small snails and perhaps capable of producing at least twice as many cercariae. Thus a single large snail may produce the same weight of infection as twenty small snails.

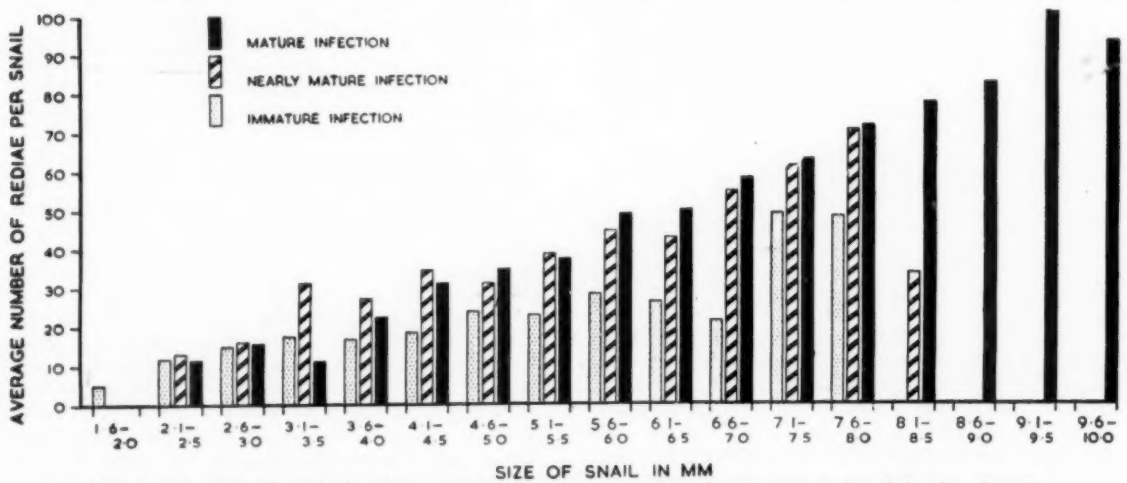

FIG. 2.—Histogram showing the relation between the average number of rediae per snail and the size of snail.

A further relevant observation is that herbage infestations have been recorded in habitats where the density of snails following repeated treatment with molluscides was so low that snails could not be found, and where the site of the habitats precluded the possibility of infection from elsewhere.

Thus, while direct evidence as to the precise relationship between the density of snails, the number of fluke eggs hatching, and the number of miracidia successfully reaching the snail is lacking, it would appear that in those areas studied—admittedly areas which are prone to outbreaks of the disease—there are enough snails necessary to produce an outbreak of the disease at all times, provided conditions are suitable for the hatching of the fluke egg and (also of great importance) for the development of the parasite within the snail.

Development of the parasite within the snail occurs when the temperature is above 10°C. (see Kendall and McCullough, 1951) and when moisture conditions maintain snail activity and growth. Under conditions of average summer temperatures, full development of the parasite within the snail will not occur in less than eight weeks, and the total time from the deposition of the fluke egg to maturation of the cercariae will take about twelve weeks.

The occurrence of ideal moisture conditions for this period of time is likely to cause also an increase in the area of colonisation of the snail and an increase in the number of snails. Increase in the area of snail habitats is a highly important factor not only in increasing the danger of infection to grazing stock but also in reducing the snail population pressure, thereby encouraging rapid growth of individual snails. An increase in the number of snails is not considered important in the epidemiology of the disease unless favourable conditions have been maintained for about five months during the period May to October, when temperature does not limit development. After this period of time snails born following the onset of favourable conditions have had time to grow to a reasonable size and, if infected, are capable of pro-

ducing a meaningful infestation on the herbage, but in summers when these conditions have occurred, e.g., 1958, it has been found that dangerous infestations on the herbage were recorded before the infection in these snails reached maturity.

Transport of the Cercaria from the Snail to the Final Host

This phase occurs in two stages—transport to the herbage followed by encystment and then transport into the final host when infected herbage is swallowed by the grazing animal. The emergence of cercariae from the snail and infection of the herbage has been considered to play an important part in the epidemiology of the disease, and the results of investigations into the stimulus necessary for the emergence of cercariae were reported by Kendall and McCullough (1951). They reported that emergence will not occur below temperatures of 9°C. and that contact with fresh water, such as occurs when it rains, stimulated emergence.

Observations in the field, however, suggest that this stage of the cycle is not as important as has been anticipated. If moisture conditions are favourable for the development of the parasite within the snail to the stage at which cercariae are ready to emerge, then inevitably the herbage will become infected. In no instances have mature infections been recorded in snails without subsequent infection of the herbage. The occurrence of a drought when snails are bearing a mature infection may delay emergence for a time, but it is considered that a delay in emergence of a week or so, depending on whether it rains or not, is unlikely to have an appreciable effect on the resulting incidence of the disease, particularly since infected herbage is sometimes not eaten in quantity until a considerable time after the herbage is infected. It is, moreover, rare to have absolute droughts in September and October, the months when the infection in the snail commonly reaches maturity.

The viability of the encysted metacercaria on the herbage has been studied by several workers. A review of the literature is given by Olsen (1947) which includes reports of metacercariae surviving from two to three hours in direct sunlight to as long as eight months in hay. With the help of my assistant, Miss Chambers, some consideration is being given to this problem at the present time. By infecting herbage, exposing it to a variety of conditions, and then feeding the herbage to rabbits, some estimate of the viability of the encysted metacercariae after varying periods of encystment may be obtained. A method of estimating viability by direct observation on metacercariae removed from herbage is also being developed. These results are as yet far from complete, but infective metacercariae have been obtained from herbage two months after encystment in summer and four months in winter.

Transport of the metacercaria to the final host is completed when the cyst is swallowed with the herbage. It has been suggested that this process may be facilitated by the recession of flood water and the drying out of dew. A dry autumn following a wet summer has also been suggested as providing favourable conditions for the ingestion of the encysted metacercariae. Although these factors may influence the infection of the final host, they are not considered to be of great importance. For instance, examination of rainfall records for Anglesey during the autumn and early winter of 1954, when a severe epidemic of the disease occurred, shows that outbreaks may be experienced when autumn and winter are particularly wet. The rainfall figures (in inches) are:

September	*October*	*November*	*December*
5·45	7·76	7·35	3·32

Under these conditions of rainfall it was extremely unlikely that flukey areas ever dried out during this period.

Of some importance in the infection of the final host is the extent to which habitats are grazed. This depends on the area of infestation compared with fluke-free grazing, the density of stocking and growth of herbage. Usually herbage in flukey areas does not appear particularly attractive to most stock, so that they tend to graze flukey areas only when herbage becomes scarce elsewhere. Under these conditions habitats may present their greatest danger to grazing stock some time after the herbage becomes infested. It is common also to find the heaviest losses occurring where the rate of stocking is high. Occasionally, however, some animals appear to prefer herbage in flukey areas. Cast ewes from hill farms sometimes graze flukey areas when first put out on to lowland pastures, whilst the occasional ewe on the hill may selectively graze flush vegetation and, as a result, die of acute fascioliasis.

Because ingestion of metacercariae by the final host is likely to extend over a long period, flukes of different ages are commonly encountered in the liver. It is, however, not always possible to assess the age of the fluke on size. Although it is usually considered that the fluke reaches sexual maturity some ten to twelve weeks after ingestion by the final host, when there is a large population of flukes in the liver, some flukes of this age may be quite small and immature. For this reason, animals which die of acute fascioliasis may have acquired their infection over a longer period of time than the accepted six to eight weeks. This fact is often of some practical importance where an investigation is being made as to the source of infection in a serious outbreak of the disease.

Conclusions and Discussion

The recurrent theme in the study of the ecology of *F. hepatica* is the importance of moisture and temperature. Unless these two factors are favourable, the life cycle is unable to occur. Each becomes a limiting factor for considerable periods of time in different seasons of the year, but, of the two, temperature is the less important, since its effect is more constant each year.

The fact that a temperature of about 10°C. (50°F.) is critical for all stages in the development of the parasite outside the final host limits development in this country from about the end of October to the beginning of May. Although temperatures fluctuate during the period May to October when development is possible, the effect of these fluctuations on the development of the parasite is not as great as might

be supposed for two reasons. First, development of the parasite both in the egg on the ground and in the snail is measured in weeks, a period sufficiently long to counter the effect of daily fluctuations from the average temperature; and, secondly, because development is possible only when moisture conditions are suitable, the very high temperatures associated with dry sunny periods do not greatly assist the development of the parasite. It is for these reasons that the effect of temperature on the development of the parasite is reasonably constant from one year to the next.

The occurrence of favourable moisture conditions during the period May to October is much more unpredictable, and it has been shown that this is essential for a period of at least three months if the fluke egg is to hatch and the parasite is to develop to a stage where the cercaria is ready to emerge from the snail intermediate host. Since infection persists quite well in æstivating snails (see Kendall, 1949b), it is not essential that suitable moisture conditions should be maintained for a continuous period of three months.

The emergence of cercariae from the snail again requires the presence of moisture, but though this process may be delayed for some little time by the occurrence of dry conditions, emergence is considered to be inevitable if moisture conditions are at all favourable for the development of the parasite. Once the herbage becomes infected, the degree of moisture, though it may influence the viability of the encysted metacercariae, appears to be of minor importance in influencing the course of infection of the final host.

Obviously, the longer moisture conditions remain favourable in flukey areas during the period May to October, the more fluke eggs are likely to hatch, the more snails become infected, the more rediae develop in the snail, and the greater the intensity and distribution of the resulting infection on the herbage. An indication of the suitability of moisture conditions in flukey areas may be obtained by determining when the soil in general is at field capacity during the period May to October. The maintenance of soil at field capacity for at least three months during this period, which is necessary before the herbage becomes infected, and for four and preferably five months before the herbage becomes dangerously infected is not a frequent occurrence in this country. Different areas of the country exhibit different patterns and, as has been reported by Ollerenshaw (1958), these go some way in explaining the varying incidence and geographical distribution of the disease in this country. This relationship between moisture, temperature and the development of the parasite has been further developed by Ollerenshaw and Rowlands (1959) in respect of the county of Anglesey in an attempt to devise a system of forecasting the incidence of fascioliasis based entirely on meteorological data.

It will be apparent by now that the incidence of the disease in any one year in this country depends primarily on the climate occurring in that year. No gradual build-up of populations of snails or the parasite is necessary as a prerequisite to an epidemic of the disease. It might be of value, therefore, to demonstrate (see Fig. 3) the relationship in time between the various stages of the life cycle which results from the interaction between the parasite and temperature, assuming that moisture conditions are favourable.

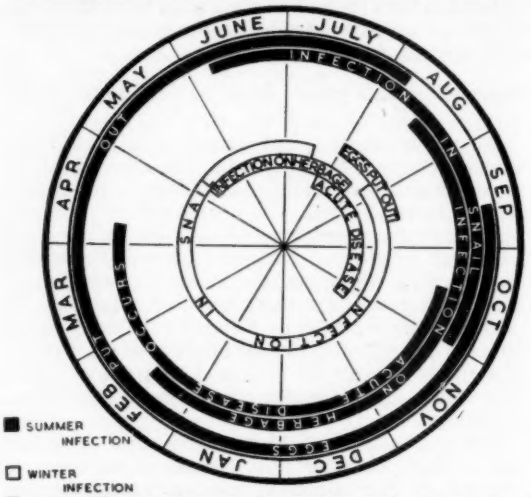

FIG. 3.—Diagram showing the timing of the life cycle of *F. hepatica* in Britain.

Fluke eggs are put on to pastures at all periods of the year. Those put out in late autumn, winter and early spring will not develop until the following early summer. These eggs, together with those put out in late spring and early summer, will hatch during the summer. The infection develops in the snail throughout summer and early autumn, and emerges on to the herbage during late summer and autumn, persisting on the herbage well into winter. This infection, called the summer infection, produces losses from the acute form of the disease which commence in October and may continue until the following spring.

Although eggs put out throughout winter may in theory contribute to this infection, our observations suggest that, in practice, they are of minor importance compared with those put out in spring and early summer, since many of them die before hatching because they are exposed to a variety of conditions for a long period before they commence to develop.

Fluke eggs put out in late summer and early autumn hatch and infect snails during autumn, but the infection is unable to develop to maturity in the snail at that time because of the onset of low temperatures. It therefore over-winters in the snail and emerges on to the herbage in the following late spring and early summer. This infection, called the winter infection, produces outbreaks of acute disease in late summer and early autumn.

The winter infection in this country is much less important than the summer infection, because the period of development of the parasite in the snail is not continuous; there is some mortality of infected snails during winter and again in early summer when mortality of parent snails commences. In some hill areas in the North of Scotland, however, the summer may be too short to permit full development of the summer infection and therefore supplements the winter infection, which may become more important than the summer infection.

Whether these infections materialise on the herbage in any year depends, of course, on the suitability of moisture conditions. The summer infection depends on conditions from May to October, the winter infection on conditions from August to October of one year and in May and June of the following year.

In this account of the ecology of *F. hepatica* in this country, the interaction between the parasite, the snail and the environment has been described in some detail, yet the end-product of this interaction—the occurrence of disease in the final host—has been given little attention. The reason for this is that, in the absence of any marked resistance or immunity to infection by the final host, the remainder of the life cycle becomes all-important. An attempt has been made in this paper to draw attention to those aspects of the life cycle of the parasite which appear most important, in the belief that their study goes some way to understanding the epidemiology of the disease.

Acknowledgments—It will be obvious to all that the observations on which this paper is based would not have been possible without the co-operation of many people throughout the country. The author would like to record his appreciation to all those who assisted him in this work. Thanks are also due to Dr. S. B. Kendall for suggestions made during the preparation of this paper.

References

KENDALL, S. B. (1949a). *J. Helminth.*, **23** (3/4), 179-90.
——. (1949b). *Ibid.*, **23** (1/2), 57-68.
——, and MCCULLOUGH, F. S. (1951). *J. Helminth.*, **25** (1/2), 77-92.
MICHEL, J. F., and OLLERENSHAW, C. B. (in press).
NEUHAUS, E. (1953). *Z. Parasitenk.*, **15** (6), 476-90.
OLLERENSHAW, C. B. (1958). *Agriculture, Lond.*, **65**, 231-5.
——, and ROWLANDS, W. T. (1959). *Vet. Rec.*, **71**, 591-8.
OLSEN, O. W. (1947). *J. Parasit.*, **33**, 36-42.
ROSS, I. C., and MCKAY, A. C. (1929). *Bull. Coun. sci. industr. Res. Aust.*, No. **43.**
THOMAS, A. P. (1883). *Quart. J. micr. Sci.*, **23**, 99-133.

THE SPEAKER'S INTRODUCTION

The Chairman, Mr. J. S. Robinson, introduced the author and invited him to present the paper. **Dr. C. B. Ollerenshaw,** in presenting his paper, said that he must thank the Organising Committee of the Congress not only for inviting him to present the paper but also for their choice of title which allowed him much freedom in ranging over the subject of fascioliasis. It should be stressed that the paper was written against the background of the serious outbreaks of the disease which had occurred the previous autumn and winter, and the areas where they had occurred. As a result, the paper was concerned mainly with ecology of liver fluke in Britain, with particular regard to conditions leading to an outbreak of the disease. The more they knew about the liver fluke in relation to its environment, the more likely they were to anticipate epidemics and the greater the value which might be derived from existing control measures, particularly those which, if they were to be fully effective, had to be fully implemented before the disease became apparent in stock.

The ultimate aim of all the work reported in the paper was the successful control of the disease. He hoped that in the discussion that day they would be able to devote some time to the problem of controlling the disease, using what was presented in the paper as a background.

The Opener

Mr. J. E. N. Sloan, the opener of the discussion, said it was more than 3 score years and 10 since Thomas and Leuckart published the results of their researches on the basic facts of the life-cycle of the liver fluke. Every few years since that time other workers in many parts of the world had contributed to their knowledge of some facet or other of the fascinatingly complex story, and since the second world war they had come to look on Weybridge as a very lively centre of research in this field. He liked to think that that interest stemmed in part, at any rate, from the events of a long week-end which the former Deputy Director of the Ministry of Agriculture, Dr. E. L. Taylor, spent at his old home in central Perthshire in the late summer of 1943. The weather was good; they had managed to save a little petrol, and they went snailing from morning till night.

But however that might be, Weybridge had greatly advanced their knowledge of liver fluke infestation in Great Britain and Dr. Ollerenshaw's paper took them yet further towards the heart of the matter. The profound influence of climate on the fortunes both of fluke and of snail intermediary had long been known, and references to individual parts of the story were to be found here and there in the literature. But now, through his long-term work in Anglesey, Dr. Ollerenshaw had been able to relate one to the other certain facts which previously seemed unrelated, and to provide a coherent synthesis which they had hitherto lacked. The influence of rainfall, of soil moisture and of temperature, the numbers of snails and their size—all those things were shown to fit neatly into the pattern of infestation both of the definitive host and of the intermediary.

It was in that sense that Mr. Sloan found the paper so helpful and valuable, the careful weighing of the facts and the critical valuation of the imponderables as they were fitted into the framework to complete the picture. Of particular interest was the point that incidence of the disease in any year in this country depended primarily on the climate occurring in that year, and that no gradual build up of populations or snail or parasite was necessary as a prerequisite to an epidemic. Presumably the old idea of "more rain, more snails, more fluke" was still valid, provided that a high proportion of the rain fell between May and October. But it might come as a surprise to most of them that, while epidemics could and did result from a general increase in snail and fluke activity after a succession of wet years, they could result also from favourable climatic conditions existing for only a few months or even weeks.

They might think from the diverging arrows of Fig. 1 that the fluke had a very difficult time and was hard put to it to complete its life cycle. Let them not be deceived! The fluke was on of the most successful parasites known and had been able to establish itself in every country in the world where suitable hosts existed or had been introduced, provided of course that the minimum temperature and moisture and requirements were available.

Mr. Sloan said he would quickly remind them from his own records just how prevalent it was in cattle in Britain. It so happened that he examined many hundreds of bovine faeces samples every year and he had notes of those positive for fluke eggs, dating back to 1947. In 5 of those years, in certain months, the number of positives exceeded 40 per cent. of the total examined. That happened in February, March and April 1947, in February and March 1948, in February, March and May 1955, in February 1956 and in March and April of the present year. Furthermore, the incidence reached 30 per cent. or more in at least one month of every year except 1950, 1953, 1957 and 1958. He hoped those high peaks fitted in with the author's climatic data, and thought they did.

Dr. Ollerenshaw made the point that there was probably always a considerable reservoir of infestation in the various mammalian hosts because many were symptomless carriers and remained untreated. The contaminating capabilities of a single carrier might be illustrated by reference to some observations he himself once made on a sheep. When it was about 5 months old it was given 50 Fasciola metacercariae. More than 6 years later, when the animal was nearly 7 years old it was slaughtered on humane grounds because it had become too heavy for its legs to carry it any more. During

all those years it was kept under conditions which precluded the possibility of reinfestation. At *post-mortem* examination 17 live flukes were recovered, so that about one-third of the original infestation was still existing after 6½ years. In fact, the fluke could live as long as its host.

For a few months after the infestation became patent the fluke egg count was about 900 e.p.g. Between 1 and 3 years it averaged about 400 e.p.g., and over the last 3 about 150 e.p.g. Spurred on by the splendid statistics of the great Norman Stoll, Mr. Sloan calculated that if all the fluke eggs produced from that sheep were laid end to end they would stretch from Berkhamsted to Beachy Head!

It might be argued that because they had drugs which were capable of giving good control of the adult flukes in the definitive host, further research into the life-cycles either of the parasite or of the vector was superfluous and mattered little. He, for one, would strongly disagree with such an attitude because he believed that drugs alone were not enough in this problem and that methods of snail control and grazing management were very important. He knew of several farms where fluke trouble had occurred and where *Lymnaea truncatula* habitats had been limited to a few square yards, sometimes in a single field, and was sure that experience could be multiplied many times over. On all those places snail elimination could be attempted with a fair prospect of success and the farms made safe, if only everybody concerned could be persuaded to apply the knowledge given into their hands by Dr. Ollerenshaw and his fellow workers on the problem.

It was true that in Britain there were 4 or 5 common species of Lymnaea and it took a little practice to gain confidence in identifying them and in getting an eye for their habitats. They were luckier in Africa where they had recently reduced the number of Lymnaea species from 50 to 2, *L. truncatula* and *L. natalensis*, so if one found a small amphibious Lymnaeid it would be *L. truncatula*, the vector of *Fasciola hepatica*, and if one found a large water-dwelling Lymnaeid it would be *L. natalensis*, the vector or potential vector of *F. gigantica*. Unfortunately, nothing quite so simple was likely in Britain, but, on the other hand, in this country one did not have *F. gigantica* to contend with and could concentrate on getting to know *L. truncatula*.

How far were the new ideas presented by Dr. Ollerenshaw likely to lead them in the direction of better fluke control? Already they had led him and Mr. Rowlands to devise a system of forecasting the probable incidence of fascioliasis. The forecasting system devised derived directly from the work being presented that day, and he (Mr. Sloan) was impressed by the close relationship established between the predicted and the actual incidence of fascioliasis over a period of several years. It bade fair to be a valuable help in tackling the fluke problem.

But neither that nor any other system of forecasting dangers to come would be of any avail unless due heed was paid to the warnings. In that connection those in general practice would have an increasingly important part to play; that of discussing the early warnings with clients and pointing out the implications of the position as it would affect them individually, and then, where necessary, advising action before it was too late. The kind of action to be taken clearly depended on the type of farm and might well involve a combination of snail control, of grazing management and of anthelmintic treatment. That last did not really fall within the purview of the present paper, but was clearly an integral part of any fluke control plan. If there was time, would the author give them extra information from his extensive experience in Anglesey and tell them something of the application of anthelmintics there?

In conclusion, there were two points Mr. Sloan wished to raise with the author. First, could he give them the best times of the year for the use of molluscicides, or was there too much variation from year to year for any standard guide; and, arising out of that, was copper sulphate still regarded as one of the cheapest and best chemicals for the job? Second, how much salinity could *L. truncatula* withstand? That question was asked in view of an experience Mr. Sloan had in the Gower Peninsula in South Wales where he found large colonies of the snail near springs on the edge of a salt-marsh and in a position which was sometimes flooded by the sea at high spring tides.

They would wish him to thank Dr. Ollerenshaw for the paper and to congratulate him on a fine contribution to their knowledge of a subject of world-wide interest. That he did with very great pleasure.

The Reply

Dr. C. B. Ollerenshaw, in reply, said that the questions had covered a wide field and if they were dealt with individually the answers might give a rather varied impression. It might be as well for him to cover a broad field and give his ideas on control, in the course of which a number of the points raised in the discussion would be dealt with. The farmer heard that there were several methods available to him. One could drain the land and thereby eradicate the snail. That was the radical solution. But often the expense of draining put that method beyond the bounds of a practical measure. The second thing one could do was to dose the stock. Perhaps in that connection carbon tetrachloride was the best known substance and in a normal year it seemed to provide reasonable control. From a farmer's point of view that method was not entirely satisfactory. It was a routine. At times it caused poisoning in animals and in a bad year the routine dosing seemed to have little effect. So the treatment of the infected stock, so far as the farmer was concerned, is not as good as it might be.

As for trying to kill the snail, at times the experts had difficulty in finding out where the snails were, so it was not to be expected that the farmer would have a precise knowledge as to where to put the molluscicides and where not to put them. On top of that, until the last year or two the farmer had to put all those measures into effect in case it happened to be a bad year.

Then there was always the question of grazing management. One could graze the non-flukey fields and rest the flukey fields. So from the farmer's point of view control of the disease was fairly difficult and required a fair amount of specialised knowledge. In most years the steps taken were of little use and, in consequence, apathy had grown up.

At the moment there appeared to be nothing brand new coming out from the point of view of control. There appeared to be no natural resistance to liver fluke by sheep and therefore one would not expect it to be possible to develop a vaccine; so they must try to make better use of the well-known measures in applying them.

Those at Weybridge had been concerned about picking out the bad year. They had heard of the 12-year cycle. The years 1920 and 1936 were bad years. It might be said that there was a bad outbreak about every 7 years. It was believed that a system had now been devised for picking out the bad year. A fair time would be required for the farmer, and perhaps the veterinary surgeon, to develop confidence in the system and then perhaps the control measures could be applied in terms of the incidence of the disease. More measures would obviously be needed for a bad year than for a good year. That feature should appeal to the farmer. He was not going to waste his money in the good year and so might be prepared to spend more in the bad year.

Assuming that the incidence of the disease could be forecast, how could the necessary measures be applied? With regard to the molluscicides, copper sulphate was still recommended. The forecast could be made at the time the infection was in the snail and before it was passed on to the herbage. It was necessary to continue dosing regularly with carbon tetrachloride. In 1928 Montgomerie demonstrated that the 1 cc. dose of carbon tetrachloride would kill a fluke only when it had been in the sheep for 10 weeks; but a 5 cc. or 10 cc. dose would kill the fluke when it had been in the liver for from 5 to 6 weeks. In a bad year more farmers were prepared to give regular doses of 5 cc. of carbon tetrachloride in 10 cc. of liquid paraffin, and they did not seem to have more losses than farmers who gave 1 cc. The usual precautions were followed.

Some people asked whether it would pay to dose up to once a week. That question could not be answered at the moment. Even on the use of well-known drugs there was still a fair amount of work to be done.

Then there was the question of drainage, about which veterinary practitioners could not do very much, though they could encourage the farmer to drain. If the farmer was thinking about controlling the disease he should be urged

first of all to assess the problem on the farm and then to decided which were the flukey areas. There was any amount of information and help available to assist those who wanted to determine the flukey areas. The farmer must next decide how far he was going to graze the flukey areas during the danger period, which lasted from September to the end of December or January. It was possible to get over the bulk of the problem of fascioliasis purely by grazing management.

In some years they might be able to recommend that hay fields, even though flukey, would be free of the disease and could augment fluke-free areas. If animals had to graze flukey fields the farmer should decide whether he could drain. Sometimes there was a habitat of only a few square yards in a field, and frequently such a habitat could be drained so that the entire field was fluke-free. If the farmer could not drain in order to give fluke-free grazing during the critical period he might be persuaded to fence off the bad spots.

The question had been raised of a forecast for the current year. It was going to be a good year and the main problem with which farmers would have to contend was controlling fluke that was picked up the year before. There were a fair number of sheep which had not been dosed since before lambing, and animals were picking up infection until lambing time and those which had done so were still carrying the infection. All sheep which were at risk the year before should be dosed, as should those with an unknown history, and also cattle. One tended to forget about cattle but they also carried the disease.

If the farmer wished to continue with routine dosing he should not be discouraged. It was necessary for a forecast to be sufficiently accurate for the farmer to apply molluscicides at the right time; but if, as often happened, the farmer desired to make a routine he should make the application twice in order to be certain of controlling the disease, in April and again in August. If only one treatment was wanted, it should be given in August.

Dr. Ollerenshaw said he had little experience of looking for snails on salt marshes and on the odd occasions when he had done so he had been unable to find any.

Eradication would be a difficult problem. It was probably just possible theoretically, but it was not a practical possibility so far as British farming was concerned. There was also a question of build-up. There was some evidence that wet weather in Britain occurred in cycles. The meteorological experts thought that 1954 was the beginning of a wet cycle, and it was a bad year for the disease, while 1955 was quite good; 1956, 1957 and 1958 were bad years. Not every year in a wet period was a bad year. The average yearly rainfall in a 7- or 11-year wet period was above that for the previous 100 years. The years, 1920, 1924, 1931, 1936, 1938, 1946, 1954 and 1958 stood out as wet years and in some cases they followed a year which was fairly dry. Dr. Ollerenshaw said he had spent many hours studying climatic data before presenting the views which were put in the paper.

The apathy of the farmer was a problem he was for ever coming up against and he had little hope to offer. It would be gratifying to hear suggested solutions from those who had much closer contact with farmers.

It had been possible to develop some eggs in faeces but on no occasion had it been possible to hatch them in faeces. It seemed correct to say that one could control the disease more easily by treatment of stock than by eradication of the snail, but treatment of stock presented a number of difficulties. The existing treatment would not kill the fluke at all ages. There were periods of the year during which stock must not be dosed. At lambing time it was thought to interfere with conception and so for 6 weeks it was stopped. There were some farmers who did not stop dosing. One farmer continued to dose half his animals and left the other half untreated, and he found that the number of ewes which aborted due to continuation of dosing was about equal to the number lost from fluke due to lack of dosing, and he was in favour of continuing to dose at lambing time.

Probably all of them knew of the substance referred to by a contributor to the discussion, which was said to have some effect on immature fluke. From the observations conducted at Weybridge it was found to kill flukes of about 5 to 6 weeks old, and in that way was comparable with carbon tetrachloride. There was no evidence of a substance which would kill flukes that were 1, 2 or 3 weeks old.

The Chairman, in proposing a vote of thanks to Dr. Ollerenshaw, said he was sure they would all wish to express their gratitude to the author and also to those who had contributed to the discussion.

Abnormal Behaviour in the Dog and Cat

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SUMMARY—Behaviour patterns in domestic dogs and cats are discussed with special reference to reproduction, the young animal, predation, eating and drinking, agonistic and social behaviour and senescence, and in appropriate circumstances comparisons are made with wild ancestors or counterparts.

Normal and abnormal behaviour are discussed in relation to experimental work involving dogs and cats and to the management and diseases of pet animals, with special reference to training, senility and the changes that may result from infectious disease and physical injury.

Introduction

THE Provisional Committee responsible for the selection of this paper preferred that its title should be restricted to abnormal behaviour, and indeed the original suggestion was that it should relate to neuroses and psychoses of these two species. Despite the undoubted existence of such conditions, from experimental as well as clinical causes, it is felt that they would at this stage scarcely provide adequate material for a paper, and that their understanding called for a discussion of normal as well as abnormal behaviour patterns. As in the human subject, the borderline between the two is not clearly defined, and it may be the situation and circumstances, some unusual or altered external stimulus, that causes apparently abnormal behaviour, not the innate or learned behaviour pattern itself.

The study of animal behaviour is developing rapidly, and I believe that it has much wider and economically more important implications in animal husbandry than studies on the neuropathics and psychopathies. The paper given by Wilkins (1957) to the Cambridge Congress, with its accompanying demonstrations, exemplified this, and outside the dog and cat field we have the problems of bull behaviour that confront the A.I. Centres.

There are available some extremely readable textbooks that present modern concepts, and I would particularly commend those of Tinbergen (1951), Thorpe (1956) and—in its relation to dogs and cats—the delightful semi-popular work of Lorenz (1954), to which numerous later references are made. Scott (1958) has much to say on dog behaviour, while Hediger's (1950 and 1955) works are of general interest to those concerned with domestic as well as with captive wild animals.

My own experience is limited largely to that of dog owner and to the use of experimental dogs for nutritional and toxicological research. On the clinical veterinary side, however, I have received considerable help from Miss Joan O. Joshua, to whom I am very greatly indebted.

Controversy over the phylogeny of the domestic dog has been revived by behavioural considerations. Darwin (1868, pp. 15-43) reviewed the evidence then available for and against a polyphyletic origin of the different domesticated races and suggested that "We shall probably never be able to ascertain their origin with certainly". Since his day, however, there have been several studies that would seem to lend a strong support to an affinity with the true northern wolf (*Canis lupus* L.) rather than with the Asiatic jackal (*Canis aureus* L.) or with some unidentified common ancestor.

Miller (1920) suggested that, on morphological grounds, there was no evidence to support affinity with the jackal, and found that the teeth of a wide range of dog skulls were strictly of the wolf type. The combined cutting and crushing type of carnassials and molars were believed by him to be highly specialised, identical with those of the northern wolf, and far from the primitive form that one would expect in a polyphyletic animal. Miller considered that superficial resemblances in general form, in colour, and in quality of fur to jackals, coyotes, foxes and other Canidæ were the basis for the erroneous hypothesis of a polyphyletic origin.

Wood Jones (1923, pp. 349-58) concluded that the Dingo, Warrigal or wild dog of Australia—to which the status of a sub-species had been given, *Canis familiaris dingo* (Blumenbach, 1780)—fell into line with all other races of domestic dogs in being of the true northern wolf type, and, moreover, that in respect of the large size of its last upper premolar, or carnassial, tooth it approached nearer to ancestral type than any other types of dogs that he had been able to examine. Following a discussion of the dental characteristics of dogs, wolves, jackals and foxes, Wood Jones wrote: "The loss of the cingulum on the first upper molar tooth is the hall-mark of the wolves—but it is the hall-mark of every breed of feral and domestic dog—and it is the hall-mark of the Dingo."

Iljin (1941), basing his conclusion on his study some years earlier of 101 wolf-dog hybrids at the Moscow Zoo Park, similarly supported a monophyletic origin of the domestic dog. He had shown that wolf-dog hybrids were fully fertile, and that in them the inheritance of eye colour, hair colour, hair pattern, ear form, body size and certain skull characters was identical with that in dogs. He could detect no essential differences in sexual behaviour, duration of pregnancy, age at which the eyes opened for the first time, eruption of the milk teeth, moulting, barking-howling behaviour (i.e., learning to bark in normal domesticated surroundings, but reversion to howling after a period of isolation), or even in what he termed nervous disposition. Usually, although not invariably, these wolf-dog hybrids were found to have only one breeding season in the year, like the wolf.

A popular review of much of this evidence has been provided by Vevers (1948). To add to it we have the work of Schenkel (1948) on emotional expression in wolves. His description, with illustrations (many of them reproduced by Thorpe, 1956), of facial attitude, of the colouring and bristling of the hair on the back, and of the use of the tail, indicate patterns of expression identical with those of domestic dogs. Indeed, the eleven forms of tail use for expression might have been portrayed direct from my own Husky, Bouncer, a veteran of the recent British Trans-Antarctic Expedition.

There have, however, been two studies, the results of which have been held by their authors to support the polyphyletic hypothesis, and each of them is based largely upon behavioural considerations. The first was that of Stockard and his collaborators (1941), who attempted to give greater definition to observed differences in the temperament of various breed crosses by use of conditioned reflex tests. They studied the parents and offspring of what were regarded as extreme behavioural types, viz., the lethargic typified by the Basset Hound and the active typified by the Alsatian or German Shepherd and the Saluki. Various blends, including extreme types of behaviour, were to be found among the cross-bred offspring, and in some instances the F_2 puppies resembled one pure breed in appearance but the other in behaviour. Stockard and his colleagues apparently believed that the many variations they produced when crossing achrondroplasic animals with those without such skeletal defects implied a polyphyletic origin, but this view seems to have received little subsequent support. Their findings must be taken into consideration with those of Whitney (1932, 1948) on the inheritance of various "aptitudes", as he termed them, useful for hunting or other types of work, and with those of Scott and his colleagues (Scott, 1950, 1954, 1958; Scott and Charles, 1954; Scott and Marston, 1950; Scott and Fuller, 1951; Pawlowski and Scott, 1956; Fuller, 1953, 1955), to some of which reference is made later, as well as with various relevant findings summarised by Burns (1952).

A much more challenging view is that advocated by Lorenz, the second of whose two popular works was made available in English in 1954 and provides a clear description of his "jackal type" and "wolf type" dogs. Lorenz is of the opinion that there are a few breeds only descended mainly, although not entirely, from the northern wolf. These breeds include Eskimo dogs, Samoyeds, Russian Lajkas and Chow-chows. He regards almost all other breeds as being derived from the Asiatic jackal, and his book contains detailed descriptions of the behaviour patterns that are the basis of his contention. Lorenz is a recognised authority on animal behaviour, and we are indebted to him for his clear descriptive matter, some of which will be utilised later in this paper. Nevertheless, there would seem to be a need for much supportive information before his views on the jackal component could find general acceptance. Relevant studies might include those on jackal-dog hybrids analogous to those of Iljin with wolf-dog crosses, but with special reference to behavioural as well as morphological characters. Reciprocal jackal-dog crosses are possible, and the hybrids are fertile in both sexes. Gray (1954), in her summary of the available evidence, states that the jackal × ♀ dog hybrids tend to resemble the jackal more than the dog in colour and conformation, but are timid and bite readily. Backcrosses to the dog are less timid and more good-natured than the F_1 hybrids. It is now fast becoming recognised that behavioural characters are of considerable importance to systematics. Nevertheless, this, like all new or relatively new applications, requires to be applied with circumspection to a given problem, and so far as possible to be taken into consideration with morphological findings. Pending further studies, it is likely that Lorenz's attractive hypothesis will not find acceptance with most authorities, notwithstanding the intrinsic value of his records of the behaviour of individual dogs and types. Scott (1958, p. 180), despite his own extensives work on dog behaviour, accepts the northern wolf origin on anatomical grounds.

From whatever wild canids the domestic dog may have been derived, it is highly probable that these were pack hunters and strictly monogamous. The fidelity of the dog fox and his excellent manners towards his vixen are models of male behaviour and constitute a problem for the fox breeder. They have been cited in relation to the old saw that two features only distinguish man from lower beasts: 'his capacity for drinking when not thirsty and his ability to make love at all seasons.' Unfortunately, or otherwise, domestication appears to have relaxed the dog's morals, and the dog breeder does not find himself faced with the same problems as the fox breeder in breaking the monogamous habit. Nevertheless, as Lorenz (1954) emphasises, male dogs behave with great chivalry towards bitches and puppies. He states that in the wolf this chivalrous self-control is extended only to females of the same pack, whereas in the breeds of dog he believes to be predominantly jackal in origin, it applies to every bitch, even if she is a complete stranger. "The Chow holds an intermediate position: if he always lives with his own kind he may behave loutishly towards strange jackal bitches, though I have never known one that actually bit them."

The relationship of mother to young is succinctly described by Thorpe (1956, pp. 396-7) as follows: "The response of the mammalian mother to her offspring and of the offspring to the mother are so intimately connected that it is difficult to consider them apart. The licking and eating of fluids and the afterbirth by a mother seems often—in dogs, cats and rats—to be a necessary preliminary to the licking and grooming of the young . . . and is probably the basis of the learned attachment of the dam to the offspring; and if this is prevented there is evidence that the usual close link may not be formed. In wild ungulates the preparturient female segregates herself; but in domestic flocks of sheep the maternal drive, which is often at full strength before the birth, may be accidentally satisfied by contact with a newborn lamb nearby before her own lamb is born. The stranger may thus be adopted and the true mother vigorously repulsed by the foster-parent while her own lamb is actually being born. But though the individual is then recognised and adopted as a result of the learning pro-

cess, the instinctive phase is shown by the powerful 'desire' to adopt and nurse something. A virgin bitch will adopt almost any object of about the right size (e.g., a bone) and lavish all her maternal care upon it for a while (Russell, 1938). Similarly, the young mammal has a strong desire to find an object for the inborn movement—pattern of sucking, and if food is supplied without the normal amount of sucking, the sucking will appear as non-nutritional. This is well seen in the persistent 'over-flow' or displacement sucking of pail-fed calves, which will suck the ears or navels of their companions or—if kept alone—their own navels or anything in their pen that offers a mouth-hold. This 'displacement' sucking perhaps also accounts to some extent for thumb-sucking in infants. It has been investigated experimentally in puppies (Levy, 1934). Puppies fed from bottles having nipples with small holes sucked for an average of eighty minutes a day, and showed no displacement sucking, whereas puppies which had been fed through nipples with holes so large that their food requirements could be satisfied in thirteen minutes a day showed post-feeding displacement sucking which continued even during sleep. Confirmation from experiments in which puppies were deprived of sucking experience, although adequately fed, during the first ten days of life has been provided by Ross (1950)." Similar evidence has been provided for kittens by Prechtl (1952).

Joshua (1959) has provided me with some extremely helpful information, based upon her own observations as veterinarian and dog-breeder, that is drawn upon freely in the paragraphs on puppy behaviour that follow. She has noted that intense respiratory stimulation, together with obvious crying, follows frictional sensations imparted by the bitch licking certain areas of the body, notably the muzzle and umbilical area. Use of this can be made in resuscitating fœtuses delivered by Cæsarean section, in that gentle friction simulating that of the bitch's tongue applied to the muzzle and umbilical area of newly-delivered puppies will intensely stimulate respiratory effort. Similarly, friction applied to the perineal area or to the preputial area in males will stimulate urination and defæcation. Clinically, use can be made of this in orphan puppies in that they can be kept completely clean by applying gentle friction to these parts, so stimulating excretions with the possibility of cleaning them up and with no soiling of the bed between feeds.

The pattern of infant behaviour is that of sleep, feeding, play. In the first week or two of life sleep takes precedence and newly-born puppies and kittens will sleep probably for 90 per cent. of the time, wakening only to feed and to be cleaned by the dam. Gradually sleep periods will become slightly less and feeding periods rather more prolonged, and, by about the third or fourth week of life, some slight evidence of a play pattern will be noted. Very shortly after the play pattern is noticed there is obvious sexual activity in that puppies at the age of three to four weeks will frequently make copulatory movements. This phase lasts only about two weeks and subsequently disappears until sexual maturity. The sucking instinct is very persistent indeed and, when puppies are three or four weeks old and the dam is leaving them for longer periods at a time, during the waking part of the time puppies will frequently suck the preputial orifice of other males in the litter.

Observation of puppies in the nest makes in clear that excretory activity follows a certain pattern. From the age of about three to four weeks, when some locomotor activity is possible, it will be noticed that immediately on waking puppies attempt to leave the sleeping area and immediately urinate. They will then feed and, having fed, will be cleaned by the dam and will then indulge in a play period. Midway through the play period it will be noted that one puppy after another suddenly becomes pensive and will again urinate and probably defæcate. Considerable use can be made of this pattern of behaviour in the house training of young puppies. Nearly all books on the training of puppies recommend that, in order to instil habits of cleanliness, puppies should be put out immediately after feeding. This would seem to be absolutely wrong. A puppy should be put out immediately after waking, should be allowed to empty the bladder, should then be brought in to feed and should be carefully watched during the subsequent play period and, immediately there is a cessation of play activity, it should be transferred to the place intended for toilet purposes, when it will probably relieve itself again. Persistence of this popular fallacy that a puppy should be clean after feeding is not based on observation of normal behaviour.

Over the first few weeks of life, sleep periods will gradually decrease and be replaced by longer and longer periods of activity. It is essential that during the immediate post-weaning period long periods of sleep should be allowed. Far too many people, on obtaining a new puppy, make no allowance for the enormous infant requirement of sleep.

Scott (1958) refers to the habit in puppies, from about three weeks of age, of tasting various odorous substances combined with mouthing of pieces of wood or pebbles, as well as the bodies of other puppies, and states that stronger reactions are given to raw meat and to the vomit of the mother, both of which are eaten without hesitation if the pup is hungry.

Scott and his colleagues have divided the developmental period in puppies into four periods, which vary from one individual to another within relatively narrow limits: viz., a neo-natal period from birth until the eyes open; a transition period from then until the animal first appears to respond to sound, at about twenty days of age; a period of socialisation lasting until final weaning at seven to ten weeks of age; and a juvenile period from then until the animal is first capable of mating behaviour proper—as opposed to the juvenile activities already mentioned—at any time from six months onwards. Until the end of the transition period the puppy cannot be made to develop conditioned reflexes or to indicate other evidence of learned responses. Electroencephalograms first indicate a difference between sleeping and waking waves at about twenty days of age, and the adult form of electroencephalogram is attained between seven and eight weeks. Similar development appears to be the case in wolf puppies, which can readily be social-

ised to human beings if taken just before the eyes open, but which if taken a few weeks later have become extremely difficult to handle. The majority of dog puppies raised under kennel conditions with but little human contact to five weeks of age will show fear reactions which, however, will gradually disappear within the next two weeks if the pups are handled regularly. Puppies taken from the litter at three or four weeks of age, and raised by hand, do not show fear reactions at five weeks, but those allowed to run wild without handling to about twelve weeks of age become increasingly timid and may be extremely difficult to catch. Such puppies can be trained, but will always be more timid and less responsive than those socialised earlier.

There are important breed differences in the intensity of this timidity or man-reaction. Scott and his co-workers (Scott and Marton, 1950; Scott and Fuller, 1951; Fuller, 1953; Scott, 1958) have studied this factor and their findings include clear demonstration that the greater shyness at five weeks of a breed such as the Basenji, compared with a breed such as the Cocker Spaniel, is inherited on Mendelian lines, there probably being two genetic factors involved. Similar differences were found to exist in respect of crouching, which appears to represent the original setting that has survived in Cocker Spaniels since there has not been any selection against it. Modern setters are really pointers.

Timidity or shyness has been studied by many other workers. The wolf-dog crosses of Iljin (1941) have already been referred to, and these included some with an extreme passive defence reaction, while Krusinskii (1938) obtained similar animals from wolf-dog crosses and from crosses of German Shepherds (Alsatians) with the Giliatski Laika breed. Thorne (1944) found that forty-three, or 73 per cent., of the F_1 and F_2 descendants of a shy, fear-biting bitch were shy, unfriendly animals, although the sires were normal friendly animals of other breeds, and that their behaviour, which appeared to be associated with a single dominant Mendelian factor, was not modifiable by training. As Burns (1952) states: "Such abnormally shy individuals also occur occasionally in pure-bred stock, and it is the general experience of breeders that excessive shyness is likely to be passed on to the progeny. This danger is particularly great in breeding from an excessively shy or nervous bitch, since, in addition to direct inheritance, the behaviour of the mother is likely to teach the pups to react as violently as she does to the presence of strangers or other disturbing influences."

Burns deals also with gun-shyness—to be distinguished from the "gun-nervousness" that is shown by dogs when first hearing shots but lost when the animals become accustomed to the gun—and cites the studies of the inheritance of grades of auditory sensitivity, by Humphrey and Warner (1934) in at least a partial explanation. Whip-shyness appears to be a similar phenomenon (Kelly, 1947). Humphrey and Warner found that stick-shyness was independent of gun-shyness, but that for both traits there were more under-sensitive males and over-sensitive bitches than would be expected if sex were not a contributory factor. Kelly advises that the shy dog intended for use with sheep should be isolated early from all other dogs.

The study of agonistic, sexual and territorial behaviour, and of dominance relationships, is of the greatest importance in training, breeding, showing, experimental studies and veterinary attention. As Joshua (1959) points out, in puppies left together in groups a tendency to fighting may be noticed from a very early age. It occurs during the play periods and appears to be a response to unusual roughness on the part of a particular member of the litter, when there will be a slight flare up of tempers, a bit of a fight and then all will settle down. So far as is possible, this tendency should be checked at an early age, if it is intended to run dogs together subsequently. This really is the childhood period when the play and fighting patterns simulate those in the human child.

Real fighting comes later and is usually linked with territorial defence or sexual considerations. Tinbergen (1942) studied the territorial behaviour of Eskimo dogs and has since (1951) summarised his findings as follows: "The Eskimo dogs of East Greenland live in packs of five to ten individuals. The members of a pack defend their group territory against all other dogs. All dogs of an Eskimo settlement have an exact and detailed knowledge of the topography of the territories of other packs; they know where attacks from other packs must be feared. Immature dogs do not defend the territory. Moreover, they often roam through the whole settlement, very often trespassing on other territories, where they are promptly chased. In spite of these frequent attacks, during which they may be severely treated, they do not learn the territories' topography and for the observer their stupidity in this respect is amazing. While the young dogs are growing sexually mature, however, they begin to learn the other territories and within a week their trespassing adventures are over. In two male dogs, the first copulation, the first defence of territory and the first avoidance of strange territory all occurred within one week."

Joshua (1959) has noted in household dogs that there may be evidence of real male aggressive behaviour as early as four to five months of age, and before there is any sign of sexual activity at all. Fighting in the female usually starts at a later stage. At about six to seven months the first signs of sexual maturity will appear in that, in the male, the typical lifting of the leg will start, as also will some degree of interest of smells outlining his own territorial area.

During adolescence obvious signs of sexual activity are not uncommon. Young dogs will ride one another regardless of sex, they will ride inanimate objects and people's legs and this behaviour must be quelled at an early age if they are to become tolerable pets. Some dogs during this period will masturbate or will have so-called "wet-dreams", the latter being particularly common in toy breeds such as the Pekingese. It is a curious fact that at this age these animals, although apparently highly sexed, may display no interest in a member of the opposite sex if it is presented for mating. As a rule these signs disappear by the time the dog is fifteen to eighteen months old.

Dominance orders are clearly manifest among dogs within litters or colonies maintained together and in the animals of a neighbourhood. Wolves set up a definite dominance relationship as they devour their prey (Scott, 1958). Among the groups of dogs studied by Scott and his colleagues, two types of biologically maladaptive behaviour were observed, one involving sexual behaviour and the other fighting. Fuller (1953) records that: "Of course, mild or mock fighting occurs in all groups and does not result in any serious harm to the participants. The criterion for maladjustment, in the four cases discussed below, is the persecution by a litter of one or more members of the group, resulting in the death of the persecuted animal, or which, in the estimation of the experimenter, would have resulted in death if the victims had not been removed. In no case were any animals torn limb from limb, but some were excluded by their litter mates from shelter (in severe weather) and died as a result of exposure. In each of these four cases, the development of the maladaptive social structure is remarkably similar. Ganging up of a group (sometimes including the mother) on a single victim occurred first during the ninth or tenth week. In one instance, the first victim managed to become a persecutor, and the final victim was an original aggressor. In three litters, the initial victim remained a victim as long as it was a member of the group. In paired dominance tests involving control of a bone, the victim tended to be subordinate, but sometimes dominated one of the persecutors when they were matched as individuals. . . . The ganging up is specifically directed against individuals, and the persecuting group lives peacefully when the victim is gone. When we have returned a victim to its own litter after a vacation, however, the attacks soon start again. The victims . . . make reasonably satisfactory adjustments when placed with strange dogs in a friendly group and, although they have been observed to be aggressive towards their new associates, group retaliation does not occur. It is the combination of specific animals which produces the maladjustment."

In dog breeding and training, aggressiveness is usually regarded as of value only if limited. In the past, some breeds were, however, selected for their ability to fight, and Scott (1958) writes: "All of the terriers are fighting breeds. They are easily excited and relatively insensitive to pain, with tough skin on their necks and shoulders and strong jaws and teeth. On the other hand, many breeds have been selected for the opposite kind of behaviour. Hounds work in packs to find game, and fighting is highly undesirable. Beagles get along peacefully together, and even strange adult males can be put in the same kennel with little more than some growling and barking."

Humphrey and Warner (1934) found that for all types of training a moderate amount of aggressiveness was desirable, and that without it there was a lack of self-confidence and 'push'. With the decreasing aggressiveness that developed in the strain they were studying, there was a correlated reduction in initiative. These authors found that the quality of "distrust" was independent of shyness, being simply an avoidance of strangers and a refusal to make friends, unconnected with fear. This quality is of value in dogs employed for army liaison work, but not in police dogs.

Lorenz (1954) has provided excellent and amusingly-illustrated accounts of encounters between dogs of different social dominance, and of one in which the two were of equally high ranking. The latter is a common sight, and from such meetings the two rivals will usually extricate themselves in a dignified manner if left well alone by attendant human beings —or bitches!

Smythe (1958) records the sad but interesting case in which a dominant hound turned upon one of its fellows, which repeated the behaviour with the next hound, and so on until the kennelman intervened with his whip. Almost immediately afterwards the pack fell upon and killed a Jack Russell terrier bitch owned by the kennelman that had hitherto regularly run with the hounds and had been on good terms with them. He records also the sudden destruction of other dogs, bitches as well as males, by individual Bull-terriers. Obviously, some releaser is responsible for this unhappy type of event, and Smythe points out that sheep-worrying may similarly occur spontaneously in animals that have hitherto led blameless lives, citing as an example a very gentle Labrador of his own. It seems certain that destruction of prey, however, is not actuated by feelings of hatred, and indeed is quite independent of fighting (see Lorenz, 1954). The sad cases of man-killing by dogs have been investigated by Grzimek (1954a, b), while Lorenz emphasises the importance of the fence, cage-bars or similar barrier in dealings not only with dogs but also with wild animals. He points out that noisy and aggressive behaviour on the part of a dog behind the fence usually subsides rapidly, to be replaced by flight, when one opens the gate, but that conversely the shy dog or wild animal may attack once the flight distance has been transgressed by stepping into the garden, room or cage. Attack may usually be thwarted by standing one's ground, and I have vivid recollections of the behaviour of a Bull-mastiff that rushed towards me as I entered by foot the drive of its home, literally skidded past me with "brakes full on" as I stood stock still, growled menacingly while I refused to budge, and suddenly accepted me to the point of accompanying me to the house. Similar experience must be common in veterinary practice.

Lorenz provides also a clear explanation of the relationship of man to the dog pack and his acceptance as a leader. He suggests that failure of the adult male wolf to accept this situation is a basal reason why it, but not the wolf bitch, is usually impossible to train as a pet, even though trained from an early age. In our own laboratory we had an interesting example of the changes that may occur with age in a Husky pup, Joey, presented to us by a member of the British North-Greenland Expedition. When young, this pup was clearly dominated by a Scottish Terrier puppy, Jack, with whom he was brought up. As he grew larger he lost no time in asserting his authority, and every morning, when the animals were let out of their runs, he would take a token grip of the back of Jock's neck to indicate his superiority. One morning, in error, he did the same to a mature Scottish Terrier, Scottie,

which reacted in no uncertain fashion to this insult. Whereupon Joey lay supine, with his neck exposed in complete surrender. Later Joey shared quarters with two Golden Retrievers, Peter and Paul, and at first made almost exaggerated efforts to indicate his subservience to them, but now that they are ageing and he is mature he attempts, not always successfully, to put them in their places. I would agree entirely with Lorenz that the most effective means of punishment is to shake the dog by the scruff of the neck. "Since dogs, like monkeys, do not hit, but bite each other on their ranking order disputes, the blow is not really an adequate or intelligible form of chastisement . . . one can imitate the penal methods of the pack-leader and involve one's own personal feelings much less by lifting the dog up by the neck and shaking him. This is the severest way I know of punishing a dog, and it never fails to make a deep impression on the offender. In actual fact, a wolf-leader which could lift up a dog of Alsatian size and shake it would be a giant, a superwolf, and, as such, the dog regards his master in the moment of chastisement. Although this form of punishment seems to us much less severe than beating with cane or whip, we must be very chary of using it, even in adult dogs, if we do not wish to intimidate them altogether."

The marking out of territory by urine is an inborn habit that has to be suppressed for satisfactory domestic relationships, but will be revived at once if that territory is invaded by a strange dog. Lorenz considers it unfair to take one's dog to a household where another dog is kept, if only in regard to the housewife's feelings at the effects upon her soft furnishings. Smythe (1958) describes, in relation to another form of releaser, unwanted urination in the house—coupled with other behaviour regarded as unwelcome: "So nowadays, when your musical sister bursts into song and Carlo, the Alsatian, lifts his head and his voice and joins in the chorus, remember that he is merely doing what his instinct warns him is his duty, and by so doing he is enabling the rest of the pack, now comfortably stretched out in their armchairs, to slumber in complete safety.

"Should some untutored pack-member, even the pack-boss, turn upon Carlo and scold him severely, the poor dog will experience embarrassment and 'conflict', a battle between what he knows he ought to do and the rebuke he has received for doing it. In order to cover up his feeling of distress, as every good pack-dog must, he will probably do something comparable with what a human being might do in similar circumstances, although the exact details of the behaviour may not be identical in the two cases. Carlo will employ what is known as a 'social-releaser' . . . (and) . . . will sit down, scratch his ribs violently with one hind foot, run around the room and possibly embarrass the whole company by cocking his hind leg against that of the table. If he is again scolded for this natural response, his nervous mechanism may go haywire, and it really is not to be wondered at that so many thoroughly respectable dogs compelled to live with very dense humans so often develop hysteria or have to be treated for 'fits'."

Urine may be voided as part of a submissive response, e.g., when a puppy is scolded or encounters a very large dog and, as Lorenz describes, as part of the process of disengagement between rivals of equal social rank. Many owners fail, of course, to recognise the submissive response of the puppy, and associate urination with excitement or scold their new pets without realising that they are receiving tokens of deference. These behavioural as well as excretory functions constitute a complication in studies on dogs housed in metabolism cages. In small cages, many dogs will not mark out their territory initially, but will hold urine often for well over twenty-four hours (Worden, 1939). The same inhibition may occur in veterinary hospitals or infirmaries, especially when the new patient is confined to small quarters. With a large metabolism cage that permits of free walking about, the difficulties are greatly lessoned (Worden and Waterhouse, 1956).

Within the age range corresponding to Scott's period of socialisation, the vast majority of puppies will in appropriate circumstances begin to form a strong attachment to an individual human being. This may persist or wane according to type—Lorenz describes clear differences between his wolf and jackal dogs in the pattern of the future relationships with man—but there is often a phase of adolescent independence when the dog will become disobedient and defiant. Discipline must be persisted with at this period, but patience also shown, and the animal will normally become more obedient again at the age of eighteen months to two years (Joshua, 1959).

Krusinskii (1946) is reported to have studied the correlation between constitution and behaviour, basing his data on 241 dogs (German Shepherd and Eskimo breeds and dogs of no specific breed) classified according to their constitutional type (eurysomic, leptosomic, "svelte" or cerebral, athletic). There was only a slight relation between degree of excitability and constitution. Leptosomic and cerebral dogs were more excitable than those with eurysomic and athletic constitutions. There was slight relation between training ability and constitution. There was slight positive correlation between active defence reaction and athletic constitution, but not between active defence and eurysomy or leptosomy. There was no correlation between constitution and passive defence.

Vaux (1953) found differences between the Dackel, Chow, Pointer and Poodle in their behaviour towards prey, and considered that some of the behavioural changes occurring during domestication had become genetically fixed breed characteristics.

The drinking pattern of the dog is of clinical and experimental interest. Tarbin (1955) has shown that dogs consume larger volumes of water daily at higher environmental temperatures by taking more frequent drinks rather than by any change in the amount of water taken at each drink. Indeed, a given dog appears to favour a characteristic size of drink that is not markedly affected by total food or water intake. It is nevertheless a fact that adult dogs with kidney damage, presumably of chronic interstitial type, will consume very long single drinks.

Dogs are equipped with remarkably good senses of hearing and smell. Engelmann (1928) found that their

sound localisation was about twice as accurate as that of man, while Kalmus (1955) has shown that the capacity of the dog to detect the individual odour of human beings is quite remarkable. This individual odour is perceived by the dog, even when mixed with another person's odour, or with some strongly smelling substances. In tracking experiments dogs were found to be able to distinguish even between the odours of identical human twin sisters.

Sight is of less importance to some canine activities than others. Vevers (1948) states that the Greyhound, Whippet, Borzoi, Saluki, Afghan, Irish Wolfhound, Scottish Deerhound, Otterhound, Elkhound and Dachshund all hunt by sight, and that the smallness of the optical angle suggests that the dog has good binocular and stereoscopic vision. Dogs are able to discriminate between differences, of size that are considerable, but are less certain with smaller differences, while their discrimination of form is poorly developed, despite the claim of some observers to have trained dogs to distinguish between squares, circles, triangles and other forms (see Warden, Jenkins and Warner, 1936).

Senile changes in dogs are very interesting and appear to follow a fairly definite pattern, not necessarily related to physical deterioration (Joshua, 1959). Hearing fails frequently in old dogs and the first sign that it is failing may be the animal's inability to detect the direction from which a sound is coming, although he can quite clearly hear the sound. This may lead gradually to total deafness, or total deafness may ensue quite suddenly. Such animals can be taught to respond to signs, but, of course, they become an increased responsibility as they have to be watched very much more carefully in relation to traffic and other hazards. Sight on the whole remains good, as does sense of smell.

Senile temperament changes become obvious in that the dog's conscience becomes slightly less active in respect of certain adult habits, particularly cleanliness. For example, dogs which have flatly refused to defæcate other than on certain specialised areas such as grass, may begin using roads or even pavements without any obvious anxiety, and in some animals even house cleanliness is lost as time goes on. These animals also, usually, become much less anxious about external stimuli; for example, dogs which have been very nervous of thunderstorms, bangs and so on become completely unperturbed as age advances. This may be associated with failing hearing, but not necessarily so.

Personality or mental changes may follow diseases which affect the nervous system; for example, hard pad encephalitis. Certain dogs, if they acquire the disease in adult life, are left with a markedly impaired intelligence. Some puppies which get a mild encephalitis during fairly early life may be left mentally retarded. Occasionally, a previously tractable, good-tempered dog will become unreliable. Similarly, temperament changes may follow accidents and, in the case of old dogs, an accident of shock which does not cause any serious physical damage may result in a rapid degeneration of mental ability. MacCurdy (1944) recorded in detail the changes in a male Dachshund, aged 4½ years, following concussion. Immediately afterwards there was a period of acute confusion and after this two discriminable types of mental abnormality. One was a general dementia not unlike senile decay in man, with loss of sexual and social drives, reduction of hunting tendencies and a general, rather seclusive, lethargy. The other was more like a "functional" human deterioration. There was regression not merely to puppy habits, but also what looked like wild canine behaviour. An interesting phenomenon was the apparent wiping out of a neurosis by the dementia, although its belated reappearance was the indirect cause of the dog's death. MacCurdy likened this to a weakening of complexes in man with the development of arteriosclerotic or senile dementia.

Jealousy and possessiveness are often displayed by dogs. Gun dogs, in particular, are extremely possessive, and may create great problems over possessions such as rubber bones and so on, when they may hold an entire family at bay. Jealousy is displayed very often if affection or notice is paid to any other animal.

Among cases of aberrant behaviour is one observed by Joshua (1959), viz., a young male adult Alsatian, a dog apparently extremely well-balanced, of excellent temperament and absolutely normal everywhere except in the back garden of the owner's house. When let out into the back garden, it behaved normally for about two minutes, would then start to look round in a thoughtful and apprehensive manner, and at that point would start chasing its tail. This it would grasp at the base, and would then chase it persistently until it fell exhausted. No reason for this behaviour could be found; it seemed unlikely that it was anything to do with the electric railway running at the bottom of the garden, but in every other place the animal's behaviour was absolutely normal.

Comben (1955) has recorded a similar case, in an otherwise healthy Airedale-type crossbred about two years old. "When he came into a room he would behave normally for a few moments, then suddenly stand rigidly with his four feet well spread out and stare fixedly at a point on the carpet as if in a trance, with not the slightest movement for one, two or up to five minutes. Then he would spring up in the air and prance upon this point, several times running. Thereafter, with hardly a moment's respite, he would move off excitedly and start the whole process again on some other point on the carpet. He would select some five or six spots all told and the whole performance would last anything up to twenty minutes."

This behaviour is said to have ended when the dog was allowed to go out on his own. "Within a week he had been seen consorting with bitches on three occasions. After ten days his strange habits in the house showed signs of abating, and after three weeks he was completely cured."

Joshua (1959) states that claustrophobia, in the Bull-terrier particularly, is not uncommon, and such dogs, if shut up in a kennel, will indulge in wild tail-chasing until they fall exhausted, or show some other similar evidence of their phobia at being shut up. It is even impossible to keep some dogs in a normal-sized room with doors and windows shut.

Another form of abnormal behaviour may be seen

in the exaggerated response of the bitch to pseudopregnancy, with nest-building (even to the extent of removing wallpaper or other suitable material) and even lactation. Bloom (1954) is careful to emphasise the range of circumstances in which pyometra may develop, but Smith and Jones (1957) state what must, I believe, be widely held among practising veterinarians, viz., that in the bitch, as in the female cat, many cases follow pseudopregnancy.

In view of the way in which stud matings are conducted, it is not surprising that there are many behavioural difficulties among both dogs and bitches. The full development of mating patterns call for appropriate surroundings and build up, and in species in which there is naturally a strong pair-bond the highly artificial nature of the stud is a considerable obstacle. Graham (1954) records that only a relatively small proportion of stud dogs form what he calls the "cave-man type", able and willing without delay to execute service with almost any bitch presented to them. All veterinarians connected with dog-breeding know the difficulties encountered in attempting to get some bitches to mate, a procedure that may call for forceful restraint. Goldstein (1957) records that there need not be such a specific act as biting the male by the bitch to keep him off, and that an individual bitch —among a colony of experimental dogs—may consistently fight off one male but readily accept the mating advances of another. Joshua (1959) mentions an interesting case of a bitch that resisted all attempts by stud dogs, but accepted a litter brother immediately. It is scarcely surprising that in some instances a pedigree bitch may fail to accept a stud dog, but present her owner with a healthy litter from what is usually termed mesalliance with a local mongrel of her choice.

Male response to a bitch in œstrus may be masked. Adie (1953) records the successful prevention of unwanted matings in a group of Huskies by the use of a proprietary preparation based on essential oils, and I have witnessed the complete deception of one of our own dogs—a crossbred terrier, Kim, whose usual reaction to bitches would place him in Graham's caveman class—when actively courted by a bitch suitably treated with chlorophyllin. He was pleased and flattered by her attentions, but quite failed to associate these with mating drive.

The response of the male dog to castration shows considerable individual variation, as the studies of Goldstein (1957) on Beagles have shown quantitatively. Some show a consistent decline in mating behaviour, comparable with that typical of rodents. Others, after an initial period of decline, showed a drive equal to, and even greater than, that before castration. The effect of castration was usually to lower the ability to lock with the bitches, i.e., the period of intromission was reduced and was presumably not accompanied by the changes necessary for locking to occur. This could be reversed by the administration of androgens.

Goldstein found that surgical removal of the pyriform cortex of male Beagles did not effect a general improvement in sex behaviour, as described for cats by Schreiner and Kling (1953), but that there was a marked increase in the speed at which these dogs mounted the bitches at the start of each test. It may seem strange to discuss dog behaviour without specific reference to the work of Pavlov (whose synthesis was made available in English in 1927) and to all the work that has followed from him, but I want merely to mention the present-day distinction between the classical conditioned reflex of the Pavlov school and trial-and-error learning (see Konorski, 1948; Thorpe, 1956).

Belief in the popularly termed "sixth sense" of the dog is widespread, and one is always coming across almost incredible stories—told usually in an attempt to demonstrate the dog's "intelligence". It must be conceded that we have not as yet adequate explanations of homing behaviour or of many other surprising canine feats. I can add one bona-fide case, in a Scottish terrier, Sheila, who was devoted to me and who remained with my parents at East Barnet from 1940 onwards, when she was seven years old, while I was working at Cambridge. As often as I could—but sometimes at intervals of some weeks—I would return home, or call in on my way to London, but my visits were usually unannounced, at different times of the day, and by any of a considerable number of local trains with which I had connected at Hatfield. Yet almost invariably Sheila would rise in pleasurable anticipation when the particular train bearing me was heard, some half a mile away, approaching the local station, and thereby inform my mother of my coming. There were over thirty stopping trains a day travelling in that direction.

The Cat

The ancestry of the domestic cat, *Felis catus* L., is also somewhat in doubt, and Pocock (1951) considered it probable that it is a composite form of which the European representatives, at all events, owe their origin to the crossing of the two wild species *Felis silvestris*, Schreber—the sub-species of which, *Felis silvestris grampia*, Miller, is the wild cat of Scotland—and *Felis lybica*, Forster. Some authorities have attempted to place these two species with *F. catus*, for they are obviously closely akin and have not any constant difference of skulls or teeth. Pocock, however, did not adopt this view, since he could not find any evidence that *F. silvestris* and *F. lybica* integrate in a wild state. He considered it probable—with due qualification—that domestic cats with *torquata* or "striped tabby" markings were hybrids between *F. silvestris* and *F. lybica*, while those with *catus* or "blotched tabby" markings were mutants of the other, there not being any recorded examples of the *catus* pattern in wild examples of either species. It is interesting that Pocock should have written: "There is indeed less to be said in favour of applying the name *catus* as a specific term to these two kinds of wild cats than for calling the wolf *Canis familiaris*." Darwin (1868, p. 45) wrote pertinently: "Whether domestic cats have descended from several distinct species, or have only been modified by occasional crosses, their fertility, as far as is known, is unimpaired." Hamilton (1896) reviewed evidence of crosses between domestic and wild cats and Peters (1932) reported on hybrids between *F. catus* and *F. silvestris* that resembled the wild parent and were heavier for their age than *F.*

catus. They were distinctly wild, spitting on handling almost before they were able to crawl. I have noted the habit, as doubtless many others must have done, in lion cubs only a few days after the eyes are opened. Pitt (1944) doubts the existence of *F. silvestris* blood in *F. catus*, but writes: "These two cats will mate and their offspring are fertile, but the hybrids show almost complete dominance of the wild-cat type. They are rusty-hued striped tabbies with thick tails ringed with black, which tails terminate more abruptly than the domestic cat; and they inherit the sporting disposition of the wild cat, besides being nervous and queer-tempered. My crossbreds could not be trusted free after they were half-grown, because they attacked poultry and ducks. Had I not shut them up, they would have been off to the woods." She states also: "The wild cat has . . . an undoubted tendency to monogamy, trait at marked variance with the promiscuous behaviour of our household puss."

Lorenz (1954) has provided beautiful descriptions of the play of kittens—activities which, as Thorpe (1956) states, provide evidence for the existence of inborn hunting behaviour. Kittens and other cats are, of course, attracted by almost any moving object. Joshua (1959) points out that since the majority of cats are neutered at an early age, and entire animals indulge in sex behaviour out of doors, the development of sexual signs and therefore of certain forms of adolescent activity are either absent or unseen. Occasionally, however, neutered males either behave like œstrous females or will ride objects in the same way as entire puppies. So far as can be ascertained, such behaviour is not associated with remnants of gonad tissue.

Leyhausen (1956) and Baron, Stewart and Warren (1957) have recently made substantial contributions to the study of social and other behaviour patterns in cats and their papers should be consulted for references to earlier studies. Leyhausen describes activities directed towards the catching, killing and eating of prey—for the release of which there are at least seven sign stimuli—and points out that the first of these are frequently directed towards substitute objects, or may even "go off *in vacuo*". Whether or not a domestic cat will kill rats is dependent upon a number of factors, mainly its "courage", which, in turn, is correlated with social rank order. Very few cats fail to be intimidated by the counter-attack of a cornered adult brown rat, and as a predator the domestic cat is specialised on much smaller rodents. Notwithstanding these findings of Leyhausen, cats may be used effectively in farm rat control (Elton, 1953). Kuo (1930) had earlier studied the development and variability of the rat-killing habit in cats, and had recorded that of twenty kittens reared alone, only nine killed rats or mice without any training, whereas eighteen out of twenty-one kittens brought up in the environment where killings were going on killed rats or mice before they were four months old. Hunger was not a factor, and cats conditioned to a vegetarian diet would kill rats, but not eat them. Leyhausen, however, considers that the whole sequence of experience from stalking to killing matures irrespective of individual experience, but that some reinforcement is usually required for the killing bite to gain its full strength. Lorenz points out the clear distinction between kitten play directed towards predation and that which stimulates fighting: "Instead of leaping on its brother as on prey—an action which may alternatively, of course, be performed—it assumes a threatening position while still galloping on, arching its back and advancing broadsides on." The fight of adult toms is described—as Lorenz says in all its complicated ceremonial—by Thompson, and Leyhausen similarly provides a graphic account. The aim—once threats have failed and actual combat begins—is always to aim at biting the nape of the adversary's neck, and so bring him down in the same way as tall prey.

Joshua (1959) finds, however, that injuries to males during fighting appear to be inflicted on repeated occasions on the same site, and that this site may be variously placed on the body, this being by no means always on the nape or head. It would seem as if fighting patterns may differ in different individuals.

Cats will defend their homes against neighbouring and intruding cats, and also possess a fairly large hunting territory which they will guard against trespassers. There may be partial overlap between neighbouring territories. An encounter between stranger cats is enacted according to fixed rules. The territory owner usually has the better of it, and as the superior cat attempts to smell the nose and anus of the intruder, it attempts to proceed with the exploration of the strange territory. Even quite young kittens will attempt a thorough exploration of a new room (Russell, 1938).

Leyhausen states that a true submissive attitude does not occur in adult cats, which as solitary animals do not establish rigid social orders if kept by numbers in a fairly large room. "One or two despots may emerge but enjoy very limited prerogatives only. One or two animals may become 'pariahs'. But neither despots nor pariahs develop of necessity, and, anyway, the rest of the crowd is on equal footing, so that the outcome of encounters cannot be predicted from the respective ranks of the animals involved. In the social intercourse of cats crowded in confinement, full intensity performances of either attack or defence behaviour are extremely rare. The single motor patterns of these types of behaviour gain a peculiar independence and appear to be at the disposal of purposive behaviour. They do not then any more express the cat's actual 'mood', but are used according to the social effect which the individual 'wants' to achieve. Low-ranking animals regularly are forced to be much more demonstrative than high-ranking ones in order to attain the same social results." The cats in our experimental colony at Huntingdon are maintained in outdoor cages, with small heated sleeping quarters, and with shelves and branches of trees to provide adequate opportunities for activities off the ground. For the most part, males and females are maintained in separate groups, and it would seem that one of their principal "wants" is to indulge in homosexual behaviour, possibly as a substitute for normal sexual activity. We have never had the slightest difficulty in breeding in captivity—quite the reverse—and Scott (1957) reports similarly on her colony at the Royal Free Hospital School of Medicine. It would seem

that the many reports of failure to breed experimental cats are due to failure to provide adequate nutrition, a suitable background for mating behaviour patterns, a control of infectious disease—sometimes, perhaps, to all three. I hope that at the end of this paper it may be possible to show a short film, for the loan of which I am indebted to Professor E. C. Amoroso, on mating behaviour in the cat. As Leyhausen states, in the course of the mating period the male is the first to take the initiative, and later it is preponderantly the female, while the partners of a pair may meet again in a long series of matings. As will be seen in the film, the female has a most pronounced "after reaction" to coitus and will violently reject any attempt by the male to repeat it too soon.

Leyhausen considers that while in more primitive carnivora, such as some Viverridæ or Mustelidæ, the male grips the female by the neck before mounting in order to block escape reactions, this has tended in the case of the domestic cat to become a ritual. Although the male may try to hold on to a female and to mount her by force, he will not be successful unless she gives in voluntarily. "In the Pantherinæ, the copulatory bite has been reduced to an accessory expression of orgasm."

Joshua (1959) points out that acquiescence, including the necessary postural changes, on the part of the female would seem to be essential for mating to occur.

Comfort (1956) made a study of longevity of cats, including six entire males of over 19 and in one case 27 years old. Mating continued throughout life in some of the oldest males investigated.

Parental care is highly developed, and, as Lorenz states, defence of the young is the one contingency in which a cat may make a prolonged and earnest attack while in the hunch-backed attitude with which it normally keeps a dog or other enemy at bay—and from which it will otherwise attack only if the "critical distance" is overstepped by its adversary. Retrieving of the young is dependent upon what many behaviourists term an "innate releasing mechanism" responding to the wail of a lost kitten.

The findings of Baron *et al.* generally confirm the asocial and individualistic behaviour of cats in relation to their fellows. They noted dominance hierarchies, but found that among cats living communally these were susceptible to change. They reported that aggression does not seem to have any direct relation to success in food getting, nor is it necessarily a concomitant of downward displacement in the dominance order, or of prolonged frustration in food getting. They consider that dominance status in the cat is not a consistent function of sex, weight, passivity or success in learning problems.

The reaction of cats to dogs and to man has received considerable study. Most people look upon cats and dogs as sworn enemies, and Spurway (1953) has produced a somewhat surprising hypothesis to account for their relationship. Nevertheless, the kitten does not seem to have any inborn understanding of the facial expressions of the dog. As Lorenz (1954) writes: "I am perfectly certain that, without previous experience, cats do not understand the expressive movements of dogs, although they are so similar to their own. Cats which are on friendly terms with dogs in the same house display a trustfulness towards strange dogs which may, and sometimes does, lead to their own ruin. I have often observed how such a cat will stare with fearless innocence full into the eyes of a strange dog which is bracing itself unequivocally for attack. It is equally unusual for a cat-friendly dog to understand the threatening of an angry cat, unless it has already learnt to do so by bitter experience. This is quite remarkable, for one would surely expect the growling of the cat to be intelligible to the dog, which expresses its emotion in just the same way." Lorenz again is responsible for a detailed account of play between a dog and cat, but reports one case only of what he regards as a true bond between members of these species, built up apparently on the basis of something akin to maternal feelings on the part of the female cat for a somewhat weak and cowardly male dog which she accompanied on walks and even defended—successfully—from attack by a strange dog.

Many cat owners are proud of the independence of their charges and Lorenz emphasises the dual role of the cat that hunts and fights out of doors but becomes a peaceful pet within. He states that, in spite of their well-defined, wild, private lives which often kept them from home for days on end, his most temperamental and virile cats were, at the same time, the most affectionate. He utters a stern warning to those who are sufficiently respected by cats for the latter to accompany them on walks: "A fully grown cat in good health and strength is unable to follow the leisurely pace of a strolling man even for half an hour without showing signs of exhaustion."

Thorpe (1956) suggests that the cat, alone among animals below the primates may possibly be capable of true imitative behaviour. He believes that most forms of apparent imitation are due to local enhancement, in which the animal's attention to a particular object or to a particular part of the environment, is drawn, and that habits acquired by trial-and-error learning on the part of pioneers (e.g., the tits that have learned to pierce the covers of milk bottles) will spread in this way. True imitation he defines as the copying of a novel or otherwise improbable act or utterance, or some act for which there is clearly no instinctive tendency. Whether or not true imitation according to this definition, the aptitude of some cats for copying is quite remarkable. I once owned a male cat, Ju-Ju, that quite spontaneously copied one of my Scottish terriers, Sandy, in begging at table, and I am quite sure that such examples are frequent. As a boy I succeeded in training young kittens, still with their mother, to stand up and box with my fingers and I well recollect going out with my father one day to find two of them indulging in a boxing match of their own. With patience, young cats will learn to play simplified forms of hide-and-seek, and it seems likely to me that in many respects their learning of tricks may differ considerably from those of the large cats familiar in the circus, training for which consists basically of the utilisation of natural and often inborn abilities (see Hediger, 1955).

Cats served extensively for the studies on learning of Thorndike, inventor of the "puzzle box" method, and of the others who followed him and severely criticised his findings, including Adams, whose string-pulling techniques helped to demonstrate the part played in insight learning by conation and perception (for references and discussion, see Russell, 1938).

Experimental surgical procedures have done a great deal to localise the centres responsible for many facets of cat behaviour (for references see Tinbergen, 1951; Goldstein, 1957; Scott, 1958). Although not strictly relevant to the present paper, the findings are of intrinsic interest and would suggest that observations on behaviour could well help to identify the site of lesions arising from accident or neurotropic infections. Many elements of the female mating pattern—raising of the pelvis, treading and contralateral swinging of the tail—may be induced by stimulation of the perinaeum in animals with the spinal cord cut at a high caudal level and kept alive by artificial respiration. It has been reported that the same response will occur in anœstrous females and even in males so treated. Male cats from which the three sacral spinal segments have been removed show aggressive sexual behaviour despite paralysis of the hind limbs, absence of sensitivity in the genital region and, of course, failure to gain intromission. Unilateral removal of the cerebral cortex does not inhibit copulatory behaviour in the male, but bilateral removal does so. Frontal lobe lesions appear to affect the motor abilities, but not the sexual excitability of males, which will follow and call to females and attempt a large number of mounts, although able to gain intromission in only a small proportion of these. Bilateral lesions of the temporal, parietal or occipital lobes has little effect upon mating, although with considerable invasion of the occipital areas there is difficulty in finding the female. In the inferior temporal lobes, bilateral injury to the amygdaloid nucleus enhances the sexual behaviour of the male, which will show a greatly increased tendency to mount females, or, indeed, other available animals such as small rabbits, dogs and chickens. This hypersexuality, as it has been called by Schreiner and Kling, can be reversed by castration but reintroduced by injections of testosterone propionate—an interesting demonstration of the interaction between neural and hormonal effects. These hypersexual males are benign, having become easier to handle and difficult to rouse to anger. Females with similar lesions likewise show signs of hypersexuality, but instead of becoming docile are inclined to a greater irritability.

Cats that have had bilateral removal of the cerebral cortex have to be fed by hand, since they cease to attempt to eat voluntarily. When picked up they tend to fly into a rage and their tendency to fight is increased.

The work of Hess and his colleagues, who have systematically probel the hypothalmic region by the introduction of minute electrodes, has localised areas where the application of a stimulus will elicit the complete behaviour patterns of fighting, eating or sleeping. All the elements of each pattern are displayed in perfect co-ordination, and the response is initiated by genuine appetitive behaviour, for the cat will seek combat, a sleeping place or food according to the area stimulated.

The early castration of male cats procludes sexual experience. In older animals the effects of castration upon sexual behaviour depend upon previous experience, those with a previous experience of mating taking much longer for their activities to decline (Rosenblatt and Aronson, 1958).

Cats, and particularly Siamese, will display resentment, very often, for example, at the introduction of another kitten or a puppy, and a common sign of such resentment is spraying, even in a neutered cat. Many neutered males will go round spraying when there has been some change which has aroused feelings of jealousy (Joshua, 1959). An excellent example of this occurred in a neutered male which had for several years been the favoured pet of a member of my staff, and which reacted in this way to the arrival of her baby.

Cats appear to suffer from shock in the nervous sense rather more readily than do dogs, and it was noteworthy during the war years that many cases of apparent shell shock in cats were encountered, whereas in the dog this was rare. Some cats became so shocked that they could not be got out from darkened corners and had to be destroyed on humanitarian grounds. There is also on record the so-called fly-catching syndrome which resulted in some cats after the severe East coast flooding (Joshua, 1959).

Cats are very subject indeed to so-called mental trauma and cases of undoubted nervous anorexia do occur. One case brought to the notice of Joshua was that of a blue Persian cat which had been given relief for rectal impaction by enemata and manipulative treatment, without general anæsthesia being induced. Although the cat made a perfectly satisfactory physical recovery, it refused to eat for some two months afterwards, became very dull and stuporous, and it was not until the cat was treated with tranquillisers, sedatives and vitamin pills that it was restored to normal. Many timid, non-aggressive cats will suffer severe mental shock after being involved in a fight with another cat, or more particularly if attacked by a dog. Although there is no physical injury, such animals frequently require treatment of the nervous system before they are restored to normal.

Lorenz states that cats always announce their intention of attacking and—"except in the case of unreliable or mentally deficient psychopaths"—never bite or scratch without giving previous unmistakable warning to the offender: usually, in fact, the gradually increasing threatening gestures are suddenly exaggerated just before action is taken.

Discussion

It has been possible to discuss a selection only of the available material, and there is a great deal of experimental work dealing with normal and abnormal behaviour—and also many clinical communications—that have been omitted from consideration or inadvertently overlooked. Nevertheless, an attempt has been made to indicate the interest and value of behaviour studies in relation not only to abnormal cases in the narrow sense but also to a better understanding

of the dog or cat as a pet, working animal, experimental subject or patient. It is to be hoped that the growing reciprocity between veterinary science and subjects such as experimental physiology will be paralleled in a similar relationship of the former to behaviour and comparative psychology. It was pleasing to read in the recent reported production of experimental atherosclerosis in dogs (Marmorston, Rosenfeld and Mehl, 1959) that one of the physiologists concerned was also a veterinarian who could readily distinguish the accompanying neurological changes from spontaneous conditions.

It is tempting to indulge in comparative considerations, and one wonders to what extent the study of aberrations, such as that shown by Smythe's Labrador, may be of help to criminologists, but we may content ourselves by quoting from Thorpe (1956) to the effect that, since Darwin's day, comparative psychology has shown ". . . the extraordinary superficial similarity between much animal and much human behaviour. This is true whether we speak of drives or orientation, of trial-and-error, of insight or of imprinting. Even such characteristically human behaviour as the framing of language and of the concept of number, the use of symbols and artistic appreciation and creation, seem no longer to offer quite the clear-cut differentiation between man and animals which was formerly supposed. And if and in so far as it proves true that the concept of purpose is necessary in the description of animal behaviour, another important similarity will have been established. In any event, it seems that ethology is already confirming the conclusions of other biological disciplines, and in doing this it may in due course provide many facts which will assist human beings in the better ordering of their social relations."

In the domestic animal field we are not faced with mass public objection to capital punishment, and while cases of the type mentioned by Smythe should surely go free, there is no justification for retention of the confirmed killer dog—be it of other dogs, cats, sheep or other animals.

I have expressed my thanks to Miss Joshua and to Professor Amoroso, and I should like to thank also Mr. Jack Pickup of Barnet, who, in the limited time available, taught me a great deal. Finally, I think that the late Dr. J. T. Edwards would have been delighted to know that this subject was to have been discussed today.

References

ADIE, R. J. (1953). *Polar Rec.*, **6**, 631.
BARON, A., STEWART, C. N., and WARREN, J. M. (1957). *Behaviour*, **11**, 56.
BLOOM, F. (1954). *Pathology of the Dog and Cat*. Evanston, Illinois: American Veterinary Publications.
BURNS, MARCA (1952). *The Genetics of the Dog*. Commonwealth Agricultural Bureaux.
COMBEN, N. (1955). *Dogs, Cats and People*. London: Thames & Hudson.
COMFORT, A. (1956). *J. Mammal*, **37**, 118.
DARWIN, C. (1868). *The Variation of Animals and Plants under Domestication*, Vol. 1. London: John Murray.
ELTON, C. (1953). *Brit. J. anim. Behav.*, **1**, 151.
ENGELMANN, W. (1928). *Ztschr. Psychol.*, 105, 317.
FULLER, J. L. (1953). *Ann. N.Y. Acad. Sci.*, 56, 214.
GOLDSTEIN, A. C. (1957). *In Hormones, Brain Function and Behaviour*, ed. H. Hoagland. London: Academic Books.
GRAHAM, R. P. (1954). *The Mating and Whelping of Dogs*. London: Popular Dogs.
GRAY, ANNIE P. (1954). *Mammalian Hybrids*. Commonwealth Agricultural Bureaux.
GRZIMEK, B. (1954a). *Ztschr. Tierpsychol.*, **10**, 71.
——. (1945b). *Zstchr. Tierpsychol.*, **11**, 147.
HAMILTON, E. (1896). *The Wild Cat of Europe*. London: R. H. Porter.
HEDIGER, H. (1950). *Wild Animals in Captivity*. London: Butterworth.
——. (1955). *Studies of the Psychology and Behaviour of Captive Animals in Zoos and Circuses*. London: Butterworth.
HUMPHREY, E. S., and WARNER, L. (1934). *Working Dogs*. Baltimore: Johns Hopkins Press.
ILJIN, N. A. (1941). *J. Genet.*, **42**, 359.
JAMES, W. T. (1951). *J. comp. physiol. Psychol.*, **44**, 71.
JOSHUA, JOAN O. (1959). Personal communication.
KALMUS, H. (1955). *Brit. J. anim. Behav.*, **3**, 25.
KELLY, R. B. (1947). *Sheep Dogs*. Sydney: Angus & Robertson.
KONORSKI, J. (1948). *Conditioned Reflexes and Neuron Organization*. Cambridge University Press.
KRUSINSKII, L. V. (1938). *Biol. Z.*, **7**, 869.
——. (1946). Cited in *Anim. Breed Abstr.*, 1949, **17**, 65 (Abstr. 240).
LEVY, D. M. (1934). *Amer. J. Orthopsychiat.*, **4**, 203.
LEYHAUSEN, P. (1956). *Verhaltensstudien an Katzen*. Berlin: Parey.
LORENZ, K. (1954). *Man Meets Dog*. London: Methuen.
MACCURDY, J. T. (1944). *Bull. anim. Behav.*, **1** (4), 28.
MARMORSON, J., ROSENFELD, S., and MEHL, J. (1959). In *Hormones and Atherosclerosis*, ed. G. Pincus. New York: Academic Press.
MILLER, G. S. (1920). *J. Mammal.*, **1**, 149.
PAVLOV, I. (1927). *Conditioned Reflexes*. Oxford University Press.
PAWLOWSKI, A. A., and SCOTT, J. P. (1956). *J. comp. physiol. Psychol.*, **49**, 353.
PETERS, H. B. (1932). *Ber. int. Ges. Erhalt. Wisents.*, **3**, 166.
PITT, FRANCES (1944). *Wild Animals in Britain*, 2nd ed. London: Batsford.
POCOCK, R. I. (1951). *Catalogue of the Genus Felis*. London: British Museum.
PRECHTL, H. F. R. (1952). *Experientia*, **8**, 220.
ROSENBLATT, J. S., and ARONSON, L. R. (1958). *Behaviour*, **12**, 285.
ROSS, S. (1950). *J. abnorm. soc. Psychol.*, **46**, 142.
RUSSELL, E. S. (1938). *The Behaviour of Animals*, 2nd ed. London: Arnold.
SCOTT, J. P. (1950). *Ann. N.Y. Acad. Sci.*, **51**, 1009.
——. (1954). *J. Nat. Cancer Inst.*, **15**, 739.
——. (1958). *Animal Behaviour*. University of Chicago Press.
——, and CHARLES, M. S. (1954). *J. genet. Psychol.*, **84**, 175.
——, and FULLER, J. L. (1951). *J. Hered.*, **42**, 191.
——, and MARSTON, M. (1950). *J. genet. Psychol.*, **77**, 25.
SCOTT, PATRICIA P. (1957). In *Handbook on the Care and Management of Laboratory Animals*, 2nd ed. by A. N. Worden and W. Lane-Petter, London: UFAW.
SCHENKEL, R. (1948). *Behaviour*, **1**, 81.
SCHREINER, L., and KLING, A. (1953). *J. Neurophysiol.*, **16**, 643.
SMITH, H. A., and JONES, T. C. (1957). *Veterinary Pathology*. London: Kimpton.
SMYTHE, R. H. (1958). *The Mind of the Dog*. London: Country Life.
SPURWAY, HELEN. (1953). *Behaviour*, **5**, 81.
STOCKARD, C. R. (1951). *American Anatomical Memoir No. 19* (with sections on behaviour by O. D. Anderson and W. T. James). Philadelphia: Wistar Institute.
TARBIN, E. J. (1955). *Amer. J. Physiol.*, **182**, 377.
THORNE, F. C. (1944). *J. genet. Psychol.*, **65**, 275.
THORPE, W. H. (1956). *Learning and Instinct in Animals*. London: Methuen.
TINBERGEN, N. (1942). *Biblioth. biotheor.*, **1**, 39.
——. (1951). *The Study of Instinct*. Oxford University Press.
VAUK, G. (1953). Cited in *Anim. breed Abstr.*, 1954, **22**, 362 (Abstr. 1711).

VEVERS, G. M. (1948). In *The Book of the Dog*, ed. B. Vesey-Fitzgerald. London: Ivor Nicholson and Watson.
WARDEN, C. J., JENKINS, T. N., and WARNER, J. H. (1936). *Comparative Psychology*, Vol. 3. New York: Ronald.
WILKINS, J. H. (1957). *Vet. Rec.*, **69**, 1397.
WOOD-JONES, F. (1925). *The Mammals of South Australia*, Part 3. Adelaide: Government Printer.
WORDEN, A. N. (1939). *Vet. Rec.*, **51**, 271.
——, and WATERHOUSE, C. E. (1956). *J. anim. Tech. Ass.*, **7**, 47.

THE SPEAKER'S INTRODUCTION

The Chairman, Mr. J. Wright, introduced the author and invited him to present his paper. **Mr. A. N. Worden,** in presenting his paper, said that in accepting the kind invitation of the Provincial Committee to present the paper, he had been concerned on account of its lack of original material. Despite the fact that he had worked with dogs and cats over many years, their normal and abnormal behaviour patterns had been variables in nutritional and toxicological experiments and not subjects for intrinsic study. It followed that he had had to depend almost entirely upon the contributions of others, and it might be added that, in view of the nature of today's discussion, the choice of material had of necessity been selective.

He was privileged to have had the opportunity of editing since its inception the scientific journal that was now the Anglo-American publication, *Animal Behaviour*, and to have acted during July, 1949, as Joint Secretary of the Round Table Conference on nomenclature in animal behaviour at Cambridge. "Définition de nos termes" was important in any branch of learning, and in the psychological sciences there was ample scope for confusion. Lay usage, or misusage, of words such as "instinct" did not simplify matters, and it was sincerely to be hoped that veterinarians who recorded cases of behavioural interest would do so with due regard to accepted terminology. Dr. W. H. Thorpe had published a report on the Cambridge Conference under the title "The Definition of Some Terms Used in Animal Behaviour Studies" in the March, 1951, issue of the *Bulletin of Animal Behaviour* (No. 9, pp. 34-40), and his remarks to the effect that "In all the discussion on the more elementary types of learning there is apt to be difficulty owing to the fact that the terms which are convenient for the physiologist tend to be misleading or clumsy for the worker dealing with the behaviour of the whole animal" were salutary.

He was indebted to is new co-editor of *Animal Behaviour*, Dr. Larry Weiskrantz of the Department of Psychology, Cambridge, for some extremely helpful comments on the paper as circulated. Dr. Weiskrantz had rightly challenged the use of the adjective "pensive" in describing the appearance of puppies prior to urination midway through the play period. It was easy even for one who was used to checking for such errors to use words in an anthropomorphic sense, and he had fallen into the same trap in writing of the "feelings" of a predator. Dr. Weiskrantz doubted also the assertion of Scott that puppies allowed to run wild until about 12 weeks of age could subsequently be trained, albeit with difficulty, while he disliked the phrase—again attributable to Scott and his colleagues—"biologically maladaptive". This came close to saying that the behaviour was "unbiological", and if in fact it meant the same as "without survival value", then there was little evidence that the behaviour described could not be shown to favour survival of the stronger members of the species. Here again, it was a question of imperfect terminology: what Scott and his colleagues had described were examples of social outcasts in dog communities, and there was little doubt that such animals existed. Those concerned with the breeding and training of dogs were all too familiar with the unfortunate problems. One of the local medical officers of health, Dr. McKellar, who was also a breeder of Boxers, had told him of "ganging-up" problems even among small groups of this breed, and that among them a bitch might be the subject of aggression by males as well as by other females. In the case of Krusinskii's work, he had had to depend upon translations of the original, and could not therefore comment adequately upon the degree of "strength" of the nervous system in his dogs. He should, however, have written in more detail about the differences between "the classical conditioned reflex of the Pavlov school and trial-and-error learning". As Thorpe (1956) had stated: "The conditioned reflex is, then, best regarded as a minute and artificially isolated piece of the learning process of the animal, and in the isolating procedure it seems that something important is lost. But the conditioned reflex is the basic element of that part of learning which consists in adjusting the innate releasive mechanism to the complexities of the external world . . . we must not expect to find that the simplest learned responses of the whole animal under natural conditions can ever be equated with a simple conditioned reflex and nothing more"; and again, "Another assumption which has proved to be erroneous is that made by many workers . . . that in the classical conditioned reflex the action pattern always remains unchanged irrespective of the unconditioned reflex which serves for reinforcement. It is now recognised that conditioned and unconditioned responses are probably never identical, and that the one is not simply a duplicate of the other. Because Pavlov measured only the salivary secretion and neglected any precise measurement of motor behaviour—in fact, because he did his best to eliminate it altogether—this essential fact was obscured".

A further point suggested by Dr. Weiskrantz related to the case of aberrant behaviour in the young Alsatian described by Joshua, namely, that there must have been something about the garden discriminable to the dog of particular significance, whether or not it was the electric railway.

He wished to record further thanks to Miss Joan Joshua. At the risk of appearing ungallant, he might say that it was some 27 years since he had come to know Miss Joshua. This had been in their pre-student days, and from her he had learned a good deal concerning dogs and their husbandry. Today he found that he could still learn from her, and the inadequacies of his paper would have been considerably greater but for her valuable contribution.

The Opener

Mr. W. B. Singleton, who opened the discussion, said that the author was to be congratulated on the way in which he had reviewed the available literature on the subject of abnormal behaviour of dog and cat. He thought he was right in saying that this was perhaps the first time that this subject had been considered by a totally veterinary audience, and although a vast amount of the paper dealt with the normal, it was quite obviously at this stage very vital that they should have a clear understanding of what was normal and was constituted abnormality. Mr. Worden had dealt with the subject extremely thoroughly, and he was to be congratulated upon his paper.

While he would agree that the study of animal behaviour was of much greater economical importance in species other than the dog and cat, nevertheless a clear understanding of fundamental canine and feline behaviour and the natural response to various conditions and circumstances should be of growing interest to clinicians and others who worked daily with these smaller species. That this study was important was emphasised by the fact that many small-animal practitioners today were becoming generally more successful as clinicians not because they had greater powers of observation but because they endeavoured to confirm diagnosis more and more by laboratory, radiography and other, for want of a better term, "artificial aids". Again, modern therapeutics, with their wide range and application, helped the clinician to obtain better and better results. The danger of those facts was obvious—they might tend to become casual and less and less observant, not of major symptoms, but of the finer points which produced a deviation from normal, either physically or mentally. This was particularly so when they were faced with a psychosomatic case, and let him say quite definitely for the sake of the doubting Thomas's who would probably be present today, that such cases did exist, but careful observation was often required in order to detect them.

The line between normal and abnormal behaviour was not easy to define, and many of the manifestations of what they believed to be abnormal behaviour might be due to environmental factors, particularly if those changed in the early

stages of mental development. Some behaviour which they now considered to be abnormal might be inherent behaviour patterns which were basic for the species or breed.

Mr. Worden's reference to Iljin's observation was interesting, particularly in connection with the point that barking-howling as opposed to barking went hand in hand with domestication. This was certainly in keeping with his own observations of the pack beagle which did not bark and was one of the most difficult dogs to domesticate, whilst the American beagle which had now been domesticated, barked. Continuing the analogy, it probably followed that the more domesticated the dog, the further it went from howling to the yap of pekes, terriers, etc.

While on this subject, it was easy to see, if one accepted Lorenz's view about the domestication of the dog, why a dog now barked; but what was the true purpose of the wolf's howl, and why did some domestic dogs revert to howling at night or when the moon was full or when a particular musical note was struck? Did we humans now consider such behaviour to be abnormal?

Did we tend to blame in-breeding too much as a cause of unfortunate nervous manifestations? He believed that they did—it was not so much a question of in-breeding as lack of culling.

The financial aspect of dog breeding was now very important. It was an industry, and many dog breeders appeared to stand with pad in hand, as a bitch was whelping, furiously assessing their profit as each puppy was born. Of course, if a veterinary surgeon was in attendance he was also standing and probably assessing his profit too! Each pup was given a potential value, and it became an obsession with most breeders to see that every puppy survived to an age when it could be sold at an optimum profit. Even though a puppy was poor physically or had a nervous disposition, too few breeders appeared to realise the harm that such puppies could do to their own breed. Taking a hard and unsentimental view, many of those puppies should be culled, but only the few really genuine breeders today appeared to have the courage to put their practical process into effect; consequently, those indifferent specimens were "protected" until many reached the age of reproduction. It was quite obvious that those individuals, which he suggested were very largely responsible for the abnormal behaviour which they observed in practice, would not survive the natural hazards of a wild state. Consequently, many breeders, from one motive or another, were seriously affecting the basic principle of the survival of the fittest and the balance of nature.

Until breeders took their heads out of the sand and faced the facts, the mental and physical standard of many breeds of dog would continue to decline. Although he sympathised with the practical difficulties involved, he maintained that it was high time that the Kennel Club took active steps to safeguard the mental and physical standards of breeds. To the best of his knowledge, they were not even giving the matter serious thought.

Turning again to the points which the author mentioned in his paper, there was little doubt that in the highly domesticated animal such as the dog the basic instincts of maternal affection and suckling had an influence upon the behaviour of some individuals in later life. Suckling gave a sense of security to both human and canine infants, and the result of this was often apparent in the poodle, for instance, which frequently showed vestigle mouth movements when under stress. Recently he had operated upon a rather temperamental miniature poodle aged 6½ years, which shared a home with its mother. The stress of the operation and the mental effect upon that poodle had caused it to attempt to suckle its dam. This behaviour had gradually disappeared as time passed and recovery from the operation took place. They might consider the behaviour to be abnormal, but was this abnormality when the manifestation was based upon a basic instinct? Mr. Worden's observations upon house training were good and sound. As veterinary surgeons were frequently asked for advice in practice, he felt that the author's observations were well worth repeating. Owners would be well advised to accept the basic pattern as described by the author and use it as a sound way of training their puppies. Certainly the problem of a puppy urinating in the house in one place only and refusing to pass water anywhere else was a great problem. He supposed that basically it was a marking out of limited territory. However, it was quite a problem in that many of these puppies, even though they were walked for hours and hours in the evening by their owners, would still perform on the doormat, and the only way of breaking this form of conditioned reflex was to take them away for 2 or 3 weeks from the particular houses in which they were living.

Mr. Worden mentioned the work of Scott and her colleagues, who divided the developmental period of puppies into 4 periods: neo-natal, 1-10 days; transition, 10-20 days; 3 weeks to 7 or 10 weeks period of socialisation, followed by the juvenile period. Of these, he felt that the period of socialisation was perhaps the most important. Scott had observed that pups which were not handled during this period were frequently nervous. This was borne out in the human, where it had been shown that babies which were given great affection during early life in the form of cuddling, maternal warmth and comfort were more contented and thrived better than those which did not receive this attention. He had observed that puppies coming from a breeder who devoted time to handling and caressing the pups were much more normal in behaviour and less likely to be nervous than those from a breeder who was too hurried to do other than attend to elementary hygiene and feeding.

Terbergen's studies of territorial behaviour in Eskimo dogs threw interesting light on the savage dog which one met in practice. It seemed that sexual maturity coincided with territory defence; taken a stage towards domestication, it was possible that the abnormally strong drive to defend a home or an owner or even an inanimate object was linked with sexual activity which might be particularly strong in the male —hence the dramatic and often highly successful results achieved by castrating these cases.

As far as early aggressiveness of puppies was concerned, he would not agree with Miss Joshua's view that this should be corrected at an early age. Apart from being a very difficult thing to correct in practice, he felt that the views of Humphrey and Warner that a moderate amount of aggressiveness was desirable were borne out in practice.

He wished to consider briefly what was probably the most common form of abnormality which they met with in practice, namely, nervous anorexia. This was particularly common in the puppy which left its mother and initial environment at an early age, e.g., 6 to 9 weeks. Also, it was more prevalent in the timid puppy. Here two points arose—the advisability of encouraging breeders to handle and play with their pups from an early age and to "bring on" any puppy in a litter which appeared to be of a retiring or nervous disposition, and secondly to encourage individual feeding of pups once they were completely weaned so that the conditioned reflex of communal feeding was broken at an early age—this he believed to be of considerable help in settling a puppy into a new home. Conditioned reflexes and extraneous factors having a direct bearing upon the mental processes of puppies and adults certainly had a very strong influence over a puppy in connection with eating, and might have an effect which transcended the physical need for food. Such cases responded well to tranquillisers in many cases, and sedatives such as phenobarbitone would have the effect of promoting appetite in practically all cases, although the treatment was limited in application because of the undesirable side effects of such drugs.

Nervous anorexia in the adult was more difficult to understand and to treat. Mrs. Brown's dog would not eat unless she powdered her nose at the same time or unless the kitchen door was left open. As a boy he well remembered a neighbour whose dog would not eat unless the wife scrubbed a floor—the house was immaculate. He had often wondered how much the conditioned reflex established so firmly in the dog was established in the other, and what would have happened if the conditioned reflex in the dog had been miraculously broken—would the owner have gone on scrubbing her floor?

This really brought him to the next very important aspect of canine behaviour which he felt warranted discussion today —the influence of a psychiatric owner upon her dog. Unfortunately, he was not a student of human psychology, and

he did not pretend to understand the inter-relation and interaction which occurred under certain circumstances between dog and owner. Nevertheless, it was obvious when one considered a few bare points that a deep and far-reaching association could develop given the necessary circumstances. First of all, there was the shifting of maternal affection of a puppy from its own mother to the two-legged one which could produce a strong connection from both points of view. In the "wolf type" dog there was in addition the inherent instinct to accept a "pack leader" which normally took the form of the human owner—thus a strong bond was created between the two. On the other side, female owners under certain circumstances responded readily to the "maternal" demands made upon them by the lonely, helpless puppy. What many people would call an unnatural association developed, the bonds of which were extremely strong. This was particularly likely to occur in the female owner who was childless, lonely, and who lacked masculine affection, and particularly if such a person lived a relatively empty life.

Thus a completely unbalanced association developed, the strength of which should never be underrated by the small animal practitioner, who should always try to deal with these circumstances with tact and understanding—not always easy in the hectic harassing days which modern life demanded of them. However, it was not difficult to appreciate that the interaction between dog and owner under these circumstances occurred readily, and nervous tension on the part of the owner was transmitted to the dog and was manifest in several ways as, for instance, nervous anorexia, abnormal timidity, and psychosomatic manifestation, such as scratching and anal gland irritation unrelated to direct physical causes. Again, such strong relationships produced mutual possessiveness which made the dog highly nervous, snappy and difficult for a stranger to handle unless removed from the presence of the owner, when they invariably became submissive and docile.

He had purposely kept his remarks general because to quote individual examples of abnormal behaviour was probably fairly easy to do, but tended to become boring after a while.

The General Discussion

Dr. F. R. Bell (R.V.C., London): "I should like to take this opportunity to congratulate Mr. Worden on reviewing this difficult subject so admirably, and Mr. Singleton for correlating the paper with the types of abnormal behaviour that are met with in veterinary practice today. There is one aspect of this problem to which the essayist has not made reference, namely, the inter-relationship between the endocrine system and the central nervous system. An example of this inter-relationship is the effect of the gonads on affective behaviour patterns. If an adult male dog is castrated then he ceases to cock his leg to urinate and reverts to the female or juvenile pattern of squatting and of expelling the urine from the bladder in a single large volume. This type of behaviour reaction can be reconverted once again to the usual male act by the injection of androgen. This example illustrates the very close interdependence of the various systems that have to do with overt behaviour. It is quite possible that this effect of hormones impinges on the central nervous system at various levels but it is likely that the hypothalamus may be the co-ordinating link."

Mr. N. Comben (Southall): "Much that has been said in this discussion about dogs applies equally well to cats. In particular, I refer to the different behaviour of many pet animals in the environment of their own homes to that exhibited in the confinement of unfamiliar kennels and catteries. We have all had experience of the animal which, though docile and easily managed at home, becomes ferociously aggressive on confinement in a cage. Such an animal frequently suffers complete nervous anorexia at the same time.

"These changes may be more marked with some breeds of cats—in particular the Siamese—than others. At a recent meeting of the Metropolitan Division of the B.S.A.V.A., where the peculiarities of this breed were discussed, I suggested that it is simply because of the more pronounced lowering of general condition and weakened resistance to infection which followed such changes, that the Siamese becomes particularly susceptible to certain virus infections in catteries. I do not believe that this breed is any more susceptible in the first place to these infections than other types of cats in their normal home environment—however bad patients they may be when they are ill.

"Such changes in the behaviour of pet animals can affect —and often make more difficult—our work with them, and any study of this nature must always be related to its application in general practice."

The Reply

Mr. A. N. Worden, in reply to the discussion, thanked Mr. Singleton for his opening remarks and for his affirmation that psychosomatic cases existed. With regard to Mr. Singleton's statements about difficulties of domestication with the beagle and the difference between British and American beagles, he thought it was most important to realise that these periods of development were of vital importance and that in fact at different stages dogs of inherently different trainability in different ways might be picked out. On the question of why domestic dogs howled, he thought that clearly some situation acted as a releaser for that type of howling, which was, of course, an innate mechanism. As for the miniature poodle which at the age of 6½ years endeavoured to suckle its mother under the stimulus of some reinforcement, he imagined that this was not so much a case of abnormal behaviour as an instance of normal behaviour being called into play by circumstances, but probably for 6½ years prior there had been an abnormal relationship in terms of pack between the two dogs. He imagined that these releaser situations must be responsible for behaviour of that type, but also for the forms of aberration which sometimes concerned things like sheep worrying and the killing of other animals, and so forth.

Various speakers, including Mr. Singleton, had referred to the desirability of a moderate degree of aggressiveness or forward behaviour in pups. He thought that most workers would agree with the majority view this afternoon that some degree of aggressiveness, provided it was not too great, was highly desirable for future training, and Lorenz believed the same to be true of cats as of dogs. Miss Joshua had pointed out that there was a time at which the pup should be taken in hand, and he reinforced the remarks made in his paper that one of the best forms of chastising a dog of any kind was not to hit or strike or kick it in a way that it did not really understand, but to shake it by the scruff of the neck.

Mr. Singleton had mentioned nervous anorexia and the difficulty of explaining its occurrence and the even greater difficulty of treating it in an adult as opposed to a puppy. He thought that possibly the main factor in these cases must be some breaking of the normal behaviour patterns. Usually these cases tended to occur either under the stimulus of kennelisation or some change, perhaps unnoticed to the owner, or routine within the home. Mr. Singleton had referred to woman/dog relationships, and these had been discussed further by Miss Freak. Obviously one was here dealing with a combination of possibly two sets of unsatisfied drives, and the combination could be deadly.

Dr. Bell had referred to the alteration of the pattern of the normal urination of the male dog under castration and its restoration with replacement therapy, and Dr. Cotchin had referred not only to the production of a feminine type of urination posture, but also to his own experience of a dog which he walked home which urinated 40 times and once failed to lift its leg. Possibly it got tired the fortieth time! But he thought that this had been almost certainly related to the confinement to other quarters, and he only wished that in the dog all effects could be explained away in relation to castration and to male hormone as, apparently, those of the urination pattern. As indicated in the paper, the effects of castration upon male dogs were extremely variable and in many cases unpredictable.

Mr. Bishop had referred to kennelisation and to the various effects of transferring dogs to such surroundings and confining them there. Of course, such dogs were often placed

in a very much more restricted territory than that to which they had been accustomed, and they were, of course, also faced with new surroundings and new reactions from them. Mere size of cage or other area in which an animal was confined had an effect on urination. They had found experimentally with dogs put into small cages that they refused to regard such a cage as territory but sat there and held their urine.

With regard to the male that appeared to become impotent or disinterested in the female after a long confinement to kennels, he thought that there was an analogy here with human experience. Despite examples to the contrary, there were reports of soldiers and other service men who had been kept entirely in male surroundings for some months or years during the war and who had in fact become impotent or completely abnormal in their relation to the opposite sex. He felt that one should perhaps think of kennels as one thought of armies or prisons in any comparative study.

Mr. Bishop's remarks reinforced the view that most dogs, at any rate, were expected to mate in highly abnormal circumstances without any prior attempt to provide conditions of build-up or of understanding the situation, and it was quite remarkable to him that there were not more failings in such matings than actually occurred.

Miss Freak had referred to the risks of carrying out a caesarean section in bitches of the hunt kennel type and the subsequent failure in such cases for normal maternal behaviour to be expressed. Miss Brancker had suggested that perhaps those circumstances which led a caesarean operation to be necessary were responsible also for the lack of maternal drive. That view might certainly be correct. He thought it was probably much more dangerous to interrupt the drive in a less socialised animal than in the case of an animal which had a more stereotyped behaviour, and he would have predicted that an animal in hunt kennel conditions would be less able to withstand interference than the animal used to family life.

Miss Freak had referred to the quasi-male behaviour of the female during urination and occasional attempts to cock a leg, and so forth. He thought that this had probably been seen under various conditions. He had seen it in circumstances where he thought it was purely an imitation of the male dog.

Miss Brancker had referred to one male whose highly active sex drive had been expressed only in respect of good-looking bitches. He could only say that one must avoid comparative interpretations!

He was sorry that he had used Miss Joshua's communications almost verbatim, but in fact he was very grateful to her for the very great help she had given him; it had enabled him to provide a paper of some sort of practical as well as theoretical interest. He agreed with Miss Joshua in her emphasis that the basis of training should be natural behaviour patterns—and not only for house training. Almost all successful forms of training for a dog as a working dog really depended upon the same principles—or should do so.

Mr. Comben and Dr. Scott had referred to the cat, and Dr. Scott had remarked that the cat was even more human-dependent than the dog. He would agree with that, but he would say that possibly the nature of the relationship between cat and human being might differ somewhat in that the cat behaved less like a pack animal than the dog. When cats were run together in groups their form of behaviour was somewhat different from that of dogs run together in groups, and he suggested that the same sort of factors operated in cat-human relationships.

He did not think that anybody had made a precise study of the development of the kitten, or at any rate had not pinned it down so precisely as Scott and his co-workers had done for puppies, and it might be that the periods were slightly different in the kitten and the puppy.

Mr. Comben had referred to upsets of animals in quarantine and in altered surroundings and to the fact that in some cases visits by the owner put the animal off food to which it had become accustomed. Again, one was tempted to use comparative explanations. This behaviour tied very closely with the behaviour of a child in hospital or a child sent to a boarding school for the first time. However, he did not wish to introduce a comparative element into the paper, since comparisons were dangerous, and obviously there was a great deal more work to be done on all aspects of this subject.

Radiobiology: the Metabolism of Iodine and Strontium in Cows

BY

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SUMMARY—Of the many fission products which could contaminate the environment following their accidental release, isotopes of iodine and strontium are considered to be the most important.

The grazing animal, by virtue of its feeding habits, may ingest considerable amounts of any radioactivity deposited on herbage and could thus be exposed to considerable danger. The dairy cow is also of vital importance as a link in the human food-chain. Many radioactive isotopes ingested by a cow, including iodine-131 and strontium-90, are secreted into its milk. A study of the metabolism of iodine and strontium in cows must therefore consider two aspects: their accumulation in the body and their secretion into milk.

A variable proportion of ingested iodine-131, roughly 15–50 per cent. depending upon the time of year, finds its way to the thyroid gland, from which it is released with a biological half-time of about twenty days. From 1·3–13·1 per cent. of a single oral dose of iodine-131 is eliminated in the milk in the seven days following administration. The factors controlling iodine secretion are not fully understood.

Strontium behaves in a manner very similar to calcium, but the animal body is capable of differentiating between the two elements. Discrimination occurs during intestinal absorption, urinary excretion and secretion into milk. Strontium is laid down in and resorbed from bone in a manner and at a rate generally similar to calcium. From 0·17–3·9 per cent. of a single oral dose of strontium-89 is eliminated in the milk in the seven days following administration. The amount appearing in the milk is directly dependent upon the milk yield.

From the experimental data available it can be concluded that, if a cow's milk is fit for human consumption, there is no danger to the cow itself from iodine-131 or strontium-90.

Introduction

THE cow at pasture occupies a unique position in its relation to environmental contamination with toxic materials of any kind. In the first place, in seeking for food it grazes a wide area and may thus collect within its rumen debris from a very large volume of air. Also, in this country the human population depends upon milk as one of its principal sources of food and, in many cases, a proportion of the toxic material eaten by a cow is excreted in its milk.

The advent of the nuclear power station and its ancillary processing plants has added to the last of industries from which toxic substances may be released accidentally into the air and contaminate the surrounding countryside. These substances, in the case of an accident involving a nuclear reactor, would be mainly radioactive products arising from the fission of the nuclear fuel. There are numerous fission products but fortunately most of them have very short half-lives (the time for the activity of a radioactive isotope to fall to half its original value as a result of disintegration of its atoms is known as the half-life), and these would not constitute a great hazard. The real danger comes from fission products with relatively long half-lives and, in particular, from substances which are absorbed into the body and which are accumulated in a particular organ or tissue.

It has been concluded that the fission products likely to provide the greatest hazard to the human population are iodine-131 (^{131}I) and strontium-90 (^{90}Sr). Iodine-131 has a relatively short half-life (eight days) but is concentrated in the thyroid gland which has a mass very small in comparison with the whole body. In the thyroid gland ^{131}I may produce local changes if present in sufficient amounts. Strontium-90 has a long half-life (probably about twenty years); it is related chemically to calcium and behaves in a very similar manner to calcium in biological systems. It thus enters into bone and remains fixed there for a very long time. In sufficient concentration it can give rise to bone cancer from irradiation of the bone itself and to leukaemia from irradiation of the bone marrow. The experimental work reported in this paper has been carried out mainly using another radioactive isotope of strontium, strontium-89 (^{89}Sr) which, because of its shorter half-life (about fifty days), is much less dangerous. Results obtained with ^{89}Sr are equally applicable to ^{90}Sr when allowance is made for the difference in their half-lives.

Both ^{131}I and ^{90}Sr are secreted into cows' milk and it has therefore been necessary to study this aspect of their metabolism. The present state of knowledge has been summarised by Garner and Sansom (1959). It is interesting to note that it has been concluded that hazards to man in the event of accidental release of fission products from a nuclear reactor would come principally through the ingestion of the milk of cows grazing pastures contaminated with ^{131}I and ^{90}Sr (Medical Research Council, Committee on Protection again Ionising Radiations, 1959).

This paper presents the results of experiments carried out at Compton to obtain some basic information on the metabolism of ^{131}I and ^{89}Sr as a preliminary to a detailed study of the toxicity of ^{131}I and ^{90}Sr to cattle. Where information on aspects not studied at Compton is available from other sources, it has been

included in order to give as complete a picture as possible at the present time.

Experimental Methods

The animals used at Compton are mature Redpoll-Ayrshire crosses. Throughout the course of the experimental period they wear a metabolism harness which allows the separate and complete collection of urine and faeces for as long a period as is necessary. They appear to suffer no discomfort in the experimental animal house, since the arrangement of the harness allows freedom of movement; their milk yield frequently increases possibly owing to the individual treatment they receive. The experimental house itself is a two-storey building with accommodation for four cows in separate stalls on the first floor, and facilities for the collection of urine, and storage, mixing and sampling of excreta on the ground floor.

Individual fission products have been administered in the form of pile-produced carrier-free material, i.e., obtained by separation from mixed fission products in a form in which all the atoms of the particular element in question are radioactive. The method of administration varied. In oral experiments it has been usual to use a solution of the isotope in a suitable chemical form either adsorbed on to a cube of cattle cake or on to a suitable adsorbent in a gelatin capsule. Intravenous injections were carried out through an indwelling catheter in the jugular or mammary vein.

It has been established that the results obtained with separated ^{131}I and ^{89}Sr are applicable to the isotopes when present in a mixture of fission products as, for example, in fall-out from weapon tests (Squire, Middleton, Sansom and Coid, 1957; Garner and Sansom, 1959).

Iodine-131 in blood, urine, milk and faeces has been assayed without pre-treatment of the samples using a well-type scintillation counter. For assay of ^{89}Sr, plasma and milk samples have been dried in thin films on large planchettes and counted directly using a β-scintillation counter with a 4-in. crystal. Faeces, urine and bone samples have been dried and ashed. ^{89}Sr and calcium precipitated with ammonium oxalate from an acid solution of the ash on to Comar plates (Comar, Hansard, Hood, Plumlee and Barrentine, 1952) and again counted using the β-scintillator.

External thyroid monitoring has been carried out using two B.12 probes (20th Century Electronics Ltd.) fixed in a rigid frame so that relatively constant positioning against the cow's neck could be achieved.

Iodine-131

Absorption and Distribution

Ingested ^{131}I is very rapidly absorbed from the cow's gut, activity appearing in the milk, for example, in less than 30 minutes after oral administration. The peak concentration in blood is reached about 5 hours after dosing. It can therefore be assumed that con-

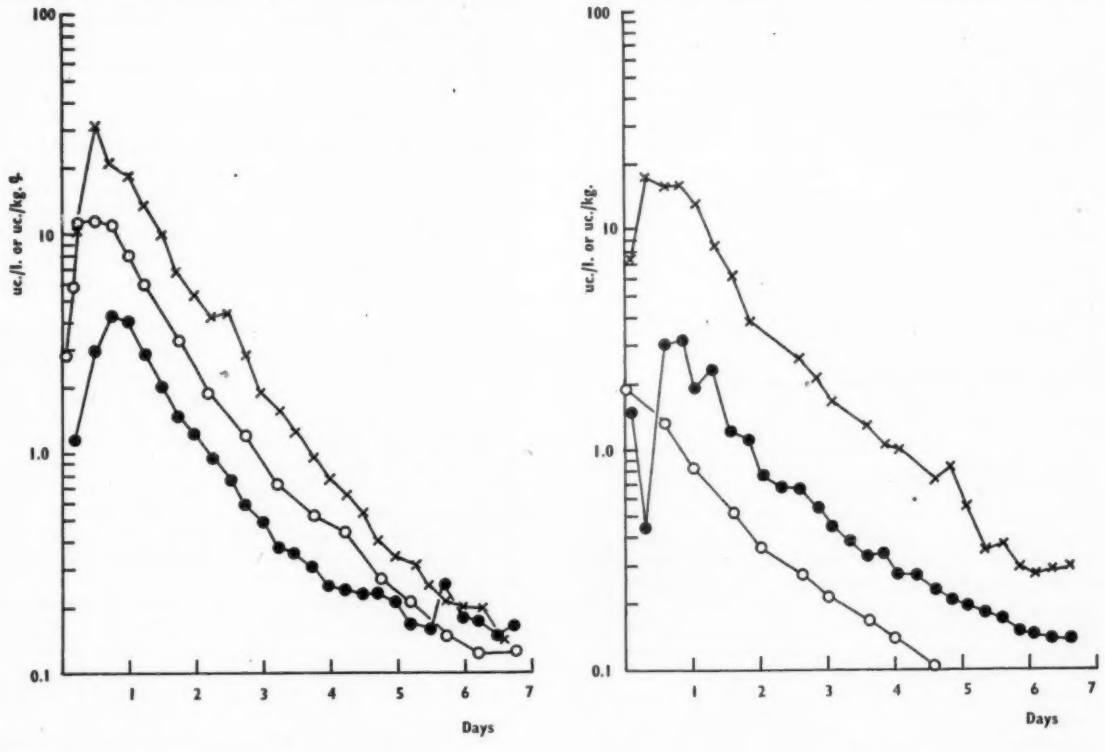

FIG. 1.—The change of concentration of ^{131}I in urine (x——x), faeces (●——●) and milk (o——o) following a single dose of 1 mc administered (a) orally (Cow No. S173), (b) intravenously (Cow No. Q42). Correction has been made for radioactive decay.

siderable absorption occurs from the rumen. In the non-ruminant animal iodine is reduced to iodide in the gastro-intestinal tract, possibly as a pre-requisite to absorption (Cohn, 1932). Since ^{131}I administered as either iodide or iodine appears to be equally available to the cow (unpublished data) and conditions in the rumen are suitable, it can be inferred that ^{131}I, however administered, is absorbed in the form of the iodide ion also in the cow.

Absorption from the gut is, for all practical purposes, complete; there is very little difference in the overall behaviour of orally and intravenously administered ^{131}I except during the first hours after dosing (Figs. 1a and b).

The distribution of ^{131}I in the tissues of the cow following a single oral dose has been studied in detail (University of Tennessee, 1954) and is shown in Table I. Concentrations significantly greater than that of the blood were limited to the contents of the gastro-intestinal tract, the faeces, urine and milk, and the thyroid and salivary gland tissue. A similar distribution has been observed in cows slaughtered some weeks after receiving multiple doses of ^{131}I (unpublished data).

TABLE I

DISTRIBUTION OF ^{131}I IN THE TISSUES OF A COW SEVEN DAYS AFTER A SINGLE ORAL DOSE (UNIVERSITY OF TENNESSEE, 1954)

Tissue	% Dose × 10^4 per g. fresh weight	% Dose in total organ
Rumen contents	0·20	—
Abomasal contents	10·1	—
Contents of small intestine	3·2	—
Contents of large intestine	2·8	—
Bile	0·52	—
Pancreas	0·36	—
Liver	0·33	0·19
Spleen	0·30	0·03
Adrenals	0·40	0·03
Thyroid	4,490	11·7
Pituitary	0·45	—
Kidney	0·46	0·05
Hair	0·11	—
Muscle	0·15	—
Udder	0·86	0·92

Iodine in the Thyroid Gland

The thyroid gland concentrates iodide from the blood by a mechanism which is not fully understood. The iodide is then oxidised, possibly to elementary iodine, before formation of iodinated amino-acids and the thyroid hormones, thyroxine and triiodothyronine. Most of the organic iodine in the gland is firmly bound as thyroglobulin. The hormones are presumably liberated by the action of a proteolytic enzyme. In the blood, thyroxine (and possibly triiodothyronine) is precipitable with the plasma proteins and is, for this reason, termed "protein-bound iodine" (PBI); it is, however, only loosely bound to protein.

If ^{131}I is administered over a period, it follows that initially it will circulate in the blood as iodide but that, after incorporation in the thyroid gland into hormones and subsequent release from the gland, a considerable proportion will be protein-bound (Fig. 2). The serum PBI level is important as an indicator of thyroid function.

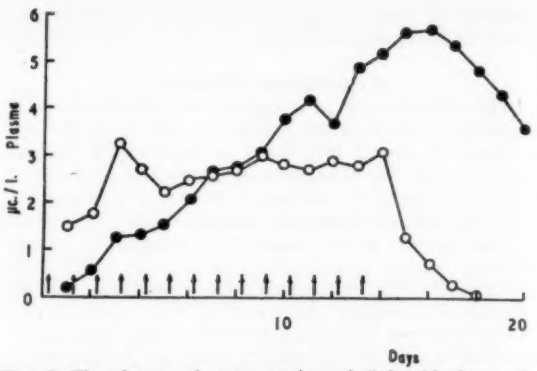

FIG. 2. The change of concentration of dialysable (o——o) and non-dialysable (●——●) ^{131}I in plasma following daily doses of 1 mc. (Cow No. T224). The arrows indicate the times of dosing.

The metabolism of ^{131}I in the thyroid gland of the cow. When a single small dose (of the order of 1 mc. or less) of ^{131}I is administered to an adult cow, the maximum uptake by the normal thyroid gland varies from about 15 to 50 per cent. of the dose, depending upon a number of factors (see below). The peak concentration is reached two to four days after dosing. The accumulated ^{131}I then gradually decreases, depending upon the turnover rate of iodine by the gland (University of Tennessee, 1955; unpublished data from this laboratory).

The retention of ^{131}I can be described by the expression:

$$R = K\,(e^{-\lambda_1 t} - e^{-\lambda_2 t})$$

where K is a constant for a given animal and describes the percentage uptake, λ_1 is the effective rate constant for elimination of ^{131}I from the gland and λ_2 is the constant describing the rate of uptake. Fig. 3 shows the curves which can be fitted to the results obtained in two groups of normal cows examined in October, 1958, and March, 1959. The equations describing retention are also given.

These results were obtained by administering a test dost of 1 mc. ^{131}I followed by external monitoring over the thyroid region. They are not corrected for ^{131}I present in soft tissues other than the thyroid gland, and K does not therefore represent a true value for the percentage uptake which is probably about 20 per cent. in the autumn and 35 per cent. in the spring. They are also not corrected for radioactive decay and the constant λ_1 and λ_2 are thus effective rate constants, i.e., the sum of the constant describing the biological processes and that describing the radioactive decay of ^{131}I.

From the constant λ_1 it is possible to obtain the effective half-life in the thyroid gland: the effective half-life is important in determining the radiation dose received by the gland from ^{131}I. In the two groups of cows studied it was 139 hours in the autumn and 126 hours in the spring.

The thyroid uptake curve in cows is similar to that in sheep, but is different from that in most other species in the much longer time to reach the maximum concentration, the flatness of the peak and the slow

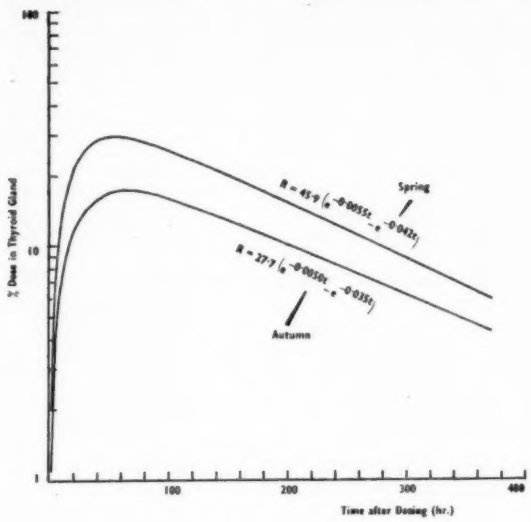

FIG. 3.—The relationship between ^{131}I concentration in the thyroid gland of lactating cows and time after dosing. The curves shown have been plotted by averaging the results of four cows. No correction has been made for radioactive decay.

loss of ^{131}I from the thyroid gland. The latter may indicate very efficient recycling of thyroid iodine in metabolism or may be due to a very slow rate of thyroid turnover.

Factors Affecting Iodine Metabolism in the Thyroid Gland. The iodine content of the thyroid gland is related to the amount of iodine in the diet, although this relationship is not simple. By increasing the supply of iodine the "storage" capacity of the system can be exceeded so that iodide enters the gland by diffusion alone. Swanson, Lengemann and Monroe (1957) found that commercial dairy feeds supplying about 10 mg. iodine daily would markedly inhibit ^{131}I uptake.

As would be expected, the rate of thyroid uptake of ^{131}I, the maximum ^{131}I content and the rate of release of ^{131}I reflect changes in thyroid activity and vary with the environmental conditions. Lengemann, Swanson and Monroe (1957) found, for example, that thyroid uptake was high in January and decreased until May, at which time low thyroid levels were attributed to a failure of the gland to accumulate iodine rather than to an increased turnover rate. Values obtained for ^{131}I uptake in January were about twice those recorded in May. As will be seen from Fig. 3, the thyroid activity of cows at Compton was considerably greater in the spring (March) than in the autumn (October). This is reflected in an increased rate of uptake, greater uptake and more rapid loss of ^{131}I from the gland.

Lodge, Lewis, Reineke and McGilliard (1958) have recorded significant differences in thyroid uptake of ^{131}I between different breeds of cattle.

In most species there seems to be a progressive but slight fall in ^{131}I uptake with age. This has been demonstrated in cattle by Amschler (1956). From the results obtained by Lodge *et al.* (1958), it appears that the uptake by five-month-old calves may be nearly twice that in adult cows.

Women show a slightly higher average ^{131}I uptake than men (Quimby, Werner and Schmidt, 1950). There is no direct evidence for a sex difference in cattle, except in so far as Sørensen (1956) found that serum PBI was greater in cows than in bullocks.

Swanson *et al.* (1957) showed that the thyroid activity of lactating cows reached a maximum in the first few weeks after parturition but that, during the later stages of lactation, ^{131}I uptake was the same as in dry cows on the same dietary regime.

Uptake of ^{131}I by the Foetus in the Pregnant Cow

In many species the placenta concentrates iodine from the maternal blood. It seems likely that such a concentrating mechanism is operative in the bovine placenta (Gorbman, Lissitsky, Michel, Michel and Roche, 1951).

Accumulation of iodine in the foetal thyroid coincides with follicle formation and is first seen at about sixty days in the foetal bovine thyroid (Gorbman *et al.*, 1951).

The concentration of ^{131}I in the foetal bovine thyroid per unit weight at term may be up to seven times greater than that in the maternal thyroid (Gorbman *et al.*, 1951).

Secretion of ^{131}I into Milk

From 1·3–13·1 per cent. (mean 5·0 per cent.) of a single oral dose is eliminated in the milk of dairy cows in the seven days following administration. Factors affecting the amount of ^{131}I transferred to milk are not understood, although the yield of milk and the season of year may be involved (Garner and Sansom, 1959).

Excretion

Elimination of iodine from the body is generally considered to take place mainly via the kidneys. From 10–56 per cent. of an oral dose of ^{131}I is excreted in the urine of the cow (Squire *et al.*, 1957, and unpublished data). Some 15–35 per cent. is excreted in the faeces (Lengemann and Swanson, 1957; Squire *et al.*, 1957, and unpublished data). Most, if not all, of the faecal iodine represents dietary iodine which has been absorbed and then re-secreted (cf. Figs. 1a and 1b).

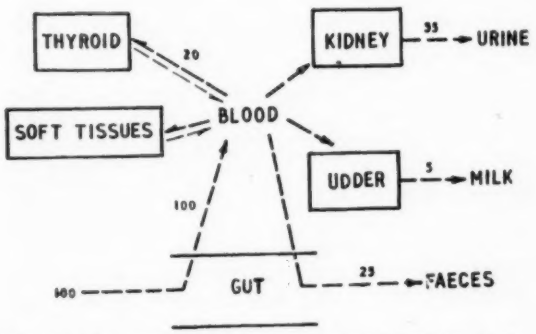

FIG. 4.—The overall fate of orally administered ^{131}I in the lactating cow.

From the distribution studies (University of Tennessee, 1954) it would appear that selective secretion occurs into the abomasum. Time disappearance curves of ^{131}I in the urine, faeces and milk are very similar (Fig. 1) and, in general, parallel that of the dialysable (non-protein-bound) fraction in the blood.

The overall fate of orally administered ^{131}I is summarised diagrammatically in Fig. 4.

Strontium-89 and Strontium-90

Strontium behaves similarly to calcium in the animal body and the ratio of ^{90}Sr to calcium in the diet is an important factor in determining the concentration which it will attain, for example, in the bones and milk. It is therefore usual to consider the metabolism of radiostrontium in relation to the quantity of calcium in the diet.

Despite the chemical similarity between strontium and calcium, the animal body is able to differentiate between the two elements. In order to compare the behaviour of strontium and calcium in biological processes, a new terminology has been developed by Comar (Comar and Wasserman, 1957). To denote the overall discrimination that is observed in the movement of the two elements from one phase to another the term "Strontium-Calcium Observed Ratio" (OR) has been introduced, thus:

$$OR_{sample-precursor} = \frac{Sr/Ca \text{ of sample}}{Sr/Ca \text{ of precursor}}$$

OR values give no information on the contribution of individual physiological processes to the discrimination if more than one process is involved. To describe the discrimination produced by an individual process the term "Strontium-Calcium Discrimination Factor" (DF) is used. By definition:

$$OR = (DF_{absorptive})\,(DF_{urinary})\,(DF_{other\ processes})$$

Absorption

Orally administered radiostrontium is probably absorbed mainly in the first half of the small intestine and absorption takes place quite rapidly. In many species differential absorption appears to be a major factor in discrimination between strontium and calcium in the body.

This discriminative process in the cow is illustrated by Fig. 5, which shows the rates of removal of ^{89}Sr and ^{45}Ca from the plasma after oral and intravenous injection. The "intravenous" curves show an almost identical magnitude and form, while, in the "oral" curves, although the rate of removal of the simultaneously administered isotopes is the same, the concentration of ^{45}Ca is about 4·5 times that of ^{89}Sr due to differences in the proportion of the isotopes absorbed from the gut.

The magnitude of the process could be measured directly if it were possible to determine the $^{89}Sr/^{45}Ca$ ratio in blood coming from the gut. This has not yet been found to be feasible but an estimate can be made if it be assumed that no discrimination occurs between the two elements when they are re-secreted into the gut. This is approximately true for the cow (see below). Making this assumption, it can be shown that:

$$DF_{absorptive} = \frac{100 - \%\text{ age of } ^{89}Sr \text{ excreted in faeces}}{100 - \%\text{ age of } ^{45}Ca \text{ excreted in faeces}}$$

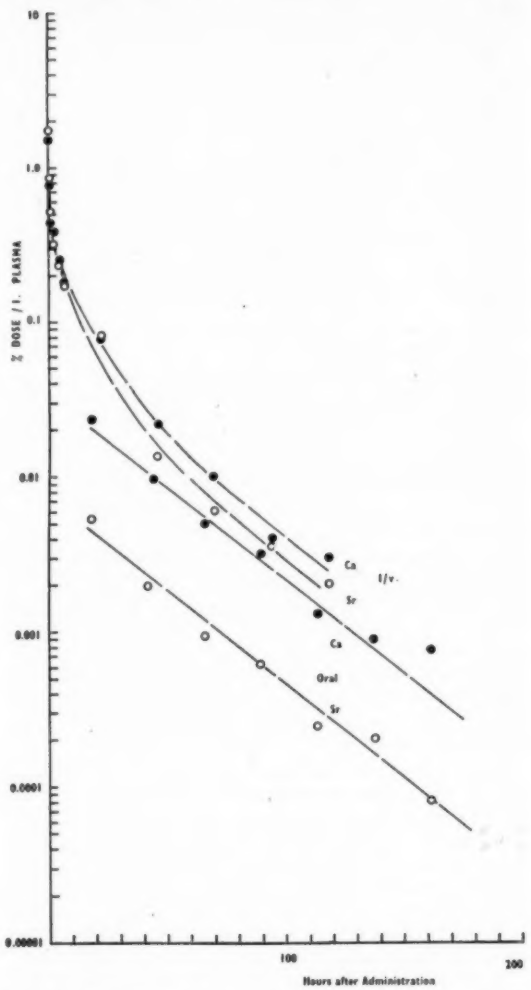

FIG. 5.—The change of concentration of ^{89}Sr (o——o) and ^{45}Ca (●——●) in the plasma of a cow (No. 80935) following oral and intravenous administration. Correction has been made for radioactive decay.

For the cow values of 0·24–0·29 have been found (unpublished data).

The fraction of an oral dose of ^{89}Sr absorbed can be calculated from the proportion excreted in the faeces if it is known what fraction of the faecal ^{89}Sr is endogenous. This information can be obtained from the faecal excretion following intravenous injection. In a range of cows it has been found that 8·0–12·4 per cent of an oral dose of ^{89}Sr is absorbed as compared with 27·6–51·9 per cent. of an oral dose of ^{45}Ca. The percentage retention in the body in these animals was 1·6–8·1 in the case of ^{89}Sr and 0·1–26·1 in the case of ^{45}Ca (unpublished data).

Strontium is absorbed more completely by the young than by the adult ruminant. In calves under one month old absorption is probably almost complete. The percentage retention of ^{89}Sr and ^{45}Ca fed

simultaneously was 69 and 77 respectively in two-month, and 3 and 17 respectively in ten-month-old calves (Comar and Wasserman, 1956). As will be evident from these figures, the gut of the very young ruminant cannot discriminate between strontium and calcium.

Milk apparently reduces absorptive discrimination, and calves on a milk diet were found to absorb both strontium and calcium to a greater extent than on a non-milk diet (Comar and Wasserman, 1956).

Effect of Dietary Calcium Level on Strontium Absorption

Theoretically it would appear possible to reduce the body burden of radiostrontium by supplementing the diet with large amounts of calcium.

Experimental studies by Comar and his colleagues (Comar and Wasserman, 1957) have shown that increasing the amount of carrier calcium in a single dose does not greatly change the amount of ^{89}Sr absorbed from the gut or retained in bone. Experiments in which high levels of calcium have been fed to laboratory animals over long periods have given equivocal results (Wasserman, Comar and Papadopoulos, 1957; Palmer, Thompson and Kornberg, 1958), but it would seem that, provided the Ca/P ratio of the diet is maintained constant, supplementing the diet with radiostrontium-free calcium would reduce the strontium burden almost proportionally.

Fate of Absorbed Strontium

It is apparent from the "intravenous" curves shown in Fig. 5 that parenterally administered ^{89}Sr and ^{45}Ca behave in the same way. The initial loss of activity from the blood is due to exchange between the blood and extra-vascular compartments; the later, slow removal is due to skeletal accumulation and excretion.

There is ample evidence to show that, by whatever route strontium is administered, it is laid down primarily in bone. A detailed description of the distribution of ^{90}Sr in the tissues of steers at various times after oral administration has been given by Comar and Wasserman (1956). Three days and eighteen months after dosing, the concentration of ^{90}Sr (in terms of per cent. dose/kg. wet weight of tissue) in muscle was 0·017 and 0·00005, and in the shaft of the femur 0·32 and 0·23 respectively.

Excretion

Over 90 per cent. of a single oral dose of ^{89}Sr is excreted in the faeces. This represents the sum of unabsorbed dietary strontium and of strontium secreted from the blood into the alimentary tract. About 16–20 per cent. (mean 18 per cent.) of absorbed ^{89}Sr is secreted in this way (unpublished data). Since about 16 per cent of intravenously administered ^{45}Ca is also excreted in the faeces, it would appear that the gut is able to differentiate to only a slight extent between strontium and calcium when they are secreted from the blood into its lumen, thus indicating that absorption and secretion involve different mechanisms.

About 1 per cent. of an oral dose of ^{89}Sr is excreted in the urine. Comparison of the $^{89}Sr/^{45}Ca$ ratio in blood and urine after intravenous injection shows that the kidney clears radiostrontium at a very much greater rate than radiocalcium. The $OR_{urine-blood}$ has been found to be 11–18 (unpublished data). Urinary discrimination is of great importance in non-ruminant animals. In ruminants, despite the large differential excretion, urinary discrimination does not contribute greatly to the overall process, since the amount of calcium excreted in the urine is very small in these species.

Strontium in Bone

Pattern of distribution. Strontium is deposited in the skeleton in all places where new bone is being laid down. Its fate depends upon the age of the animal at the time of deposition.

The distribution of radiostrontium has been studied in the greatest detail in the rabbit skeleton (Kidman, Rayner, Tutt and Vaughan, 1952; Jowsey, Rayner, Tutt and Vaughan, 1953; Jowsey, Owen, Tutt and Vaughan, 1955). Autoradiographs of the bones of steers (Comar and Wasserman, 1956) indicate that the pattern of strontium deposition and resorption is essentially the same in cattle.

In very young animals radiostrontium is concentrated beneath the epiphyseal plate and in certain parts of the shaft of the long bones. The localised areas soon disappear and, after a few weeks, the isotope is diffusely distributed throughout the bone. This redistribution is due to release of strontium from the bone; some of it is excreted and some of it is taken up again from the blood and incorporated into newly formed bone.

In rather older animals, but before growth in length of the long bones ceases, the areas of concentration in the shaft persist because the growth in diameter of the shaft is not sufficient to lead to resorption of bone salts from these sites.

In mature animals strontium appears to be diffusely distributed throughout the bones, although histological examination reveals that there are again local areas of concentration in building sites for, of course, there is constant resorption and excretion of bone salts throughout life, although the processes are slow.

Metabolism of radiostrontium in bone. The overall metabolism of strontium in bone follows closely that of calcium. It is believed that strontium is deposited by ionic exchange on the surface of bone crystals and probably penetrates into the surface of the crystals by a process of diffusion. It is incorporated in the sites of active calcification probably in the form of a strontium phosphate-carbonate complex and is removed from such areas by the normal processes of resorption. Kidman *et al.*, 1952; Tutt, Kidman, Rayner and Vaughan, 1952.)

Radiostrontium is only very slowly lost from the adult skeleton. In cattle it has been shown that the ^{90}Sr content of bone after exposure to a single dose decreased only by a factor of about 3 over a period of 18 months (Comar and Wasserman, 1956). The turnover rate of ^{90}Sr was found by Comar and his colleagues to vary considerably in different bones and in different parts of the same bone. As would be expected, the epiphyses of the long bones take up more strontium than the shaft but lose it more rapidly. In young animals strontium appears to be more or

less evenly distributed between all bones of the skeleton.

From a comparison of the blood and bone $^{89}Sr/^{45}Ca$ ratios following intravenous administration, it can be concluded that the skeleton of the cow does not differentiate appreciably between the two isotopes.

An attempt has been made to compare the rates of exchange, accretion and resorption of ^{89}Sr and ^{45}Ca in lactating cows in the whole body and in the tail bones (unpublished data). There are difficulties in interpreting the results, but the general conclusion to be drawn is that strontium and calcium are laid down and resorbed from bone at much the same rates.

Insufficient information is available to be able to obtain an accurate picture of the long-term pattern of retention of radiostrontium in the cow's skeleton.

Passage of radiostrontium to the foetus in the pregnant animal. More calcium than strontium passes the placental barrier from the dam to the foetus. In experiments in rats and rabbits (Wasserman, Comar, Nold and Lengemann, 1957) the $OR_{foetus-maternal\ plasma}$ was found to be 0·65 and 0·49 respectively, indicating that the placenta is about twice as permeable to calcium as to strontium. No direct information is available on placental discrimination in the cow.

In animals receiving an adequate supply of dietary calcium only a very small amount of radiostrontium stored in the maternal skeleton passes to the foetus: there is no dramatic mobilisation of strontium from the mother's bones during pregnancy. This would not be true of animals whose calcium status was poor.

Secretion of Radiostrontium into Milk

From 0·17–3·9 per cent. (mean 0·8 per cent.) of a single oral dose of ^{89}Sr is eliminated in the milk of a dairy cow in the seven days following administration. There is a wide variation between individual animals which can be accounted for by differences in the milk yield (Garner and Sansom, 1959).

There is a considerable reduction in the Sr/Ca ratio during passage of the two elements from the circulating blood to the milk. In a series of experiments the $OR_{milk-plasma}$ in the cow has been found to average 0·35 (Comar, Russell and Wasserman, 1957; unpublished data from this laboratory).

Mammary discrimination is of considerable importance in the lactating ruminant, tending to reduce the Sr/Ca ratio in the milk and to raise the Sr/Ca ratio in the body (see below).

Comparison of the Overall Metabolism of Strontium and Calcium in the Lactating Cow

The overall fate of a single dose of ^{89}Sr or ^{45}Ca administered orally to a lactating cow is shown diagrammatically in Fig. 6. The figures are means obtained from balance experiments on four cows. The rather high proportion of ^{89}Sr eliminated in the milk is accounted for by the fact that these animals gave considerably more milk than the average cow used at Compton.

Table II shows typical values for the observed ratios and discrimination factors obtained in these experiments. The various factors have been calculated on the basis of equations developed by Wasserman, Lengemann and Comar (1958).

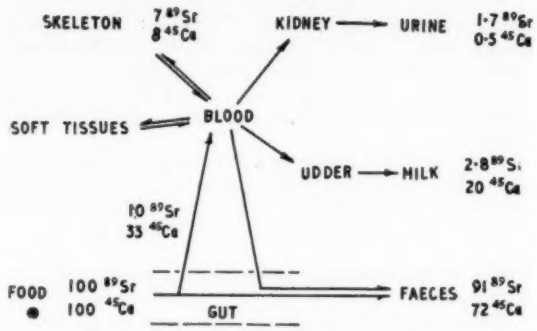

FIG. 6.—The overall fate of orally administered ^{89}Sr and ^{45}Ca in the lactating cow.

TABLE II

STRONTIUM-CALCIUM OBSERVED RATIOS (OR) AND DISCRIMINATION FACTORS (DF) IN A LACTATING COW (No. 80935)

$OR_{plasma-diet}$	0·24
$OR_{milk-plasma}$	0·46
$OR_{milk-diet}$	0·11
$OR_{urine-plasma}$	18·3
$OR_{urine-diet}$	4·6
$DF_{absorptive}$	0·24
$DF_{urinary}$	0·63
$DF_{lactational-milk}$	0·74
$DF_{lactational-body}$	1·15

It is clear that preferential absorption of calcium from the gut is the major discriminatory process in the ruminant animal. Urinary discrimination is of little significance because of the small proportion of strontium and calcium eliminated in this way, and this fact, together with the considerable lactational discrimination in favour of calcium secretion into milk, makes the overall picture in the lactating cow the reverse of that in the non-lactating non-ruminant animal. In the latter there is a tendency for discrimination processes to reduce the Sr/Ca ratio in the body; in the lactating ruminant the effect of mammary discrimination is apparently so great as to increase the Sr/Ca ratio.

The overall discrimination between the two elements during their passage from the diet to the milk (by a factor of about 10 in favour of calcium) is of considerable importance, since it reduces the ratio of radiostrontium to stable calcium in one of the principal items of the human diet.

Conclusions

From the basic information now available, it is possible to predict, in the case of ^{131}I, both the radiation dose which would be received by the thyroid gland of a cow grazing herbage contaminated with the isotope and the concentration of ^{131}I likely to be found in the cow's milk. Direct estimates of the toxicity of ^{131}I to cows (unpublished) have shown that the minimum radiation dose producing any observable change in the thyroid gland is probably about 10,000 rads. The amount of ^{131}I which must be ingested by a cow to deliver this radiation dose to the gland can be shown to be about 75 times that which

would have to be ingested to give the maximum permissible concentration in milk for a human infant under emergency conditions according to the latest recommendations (Medical Research Council, Committee on Protection again Ionising Radiations, 1959).

In the case of ^{89}Sr and ^{90}Sr, insufficient data are available for a direct assessment to be made of their toxicity to the cow. An approach can be made by extrapolation from the results of toxicity trials in other species. It is not possible in the present paper to consider the assumptions and calculations involved, but it can be stated that, as in the case of ^{131}I, if a cow's milk is considered safe for human consumption, the animal itself is in no danger from the ^{89}Sr or ^{90}Sr it has consumed.

References

AMSCHLER, J. W. (1956). *Ztschr. Tierzücht Züchtungsbiol.*, **67**, 305.

COHN, B. N. E. (1932). *Arch. intern. Med.*, **49**, 950.

COMAR, C. L., HANSARD, S. L. HOOD, S. L. PLUMLEE, M. P., and BARRENTINE, B. F. (1951). *Nucleonics*, **8**, 19.

——, RUSSELL, R. S., and WASSERMAN, R. H. (1957). *Science*, **126**, 485.

——, and WASSERMAN, R. H. (1956). In *Progress in Nuclear Energy, Series VI, Biological Sciences*, vol. 1. London: Pergamon Press.

——, —— (1957). International Conference on Radio-Isotopes in Scientific Research, Paris. Paper No. UNESCO/NS/RIC/176.

GARNER, R. J., and SANSOM, B. F. (1959). *Vet. Rec.*, **71**, 670.

GORBMAN, A., LISSITSKY, S., MICHEL, O., MICHEL, R., and ROCHE, J. (1951). *C.R. Soc. Biol. (Paris)*, **45**, 1642.

JOWSEY, J., OWEN, M., TUTT, M., and VAUGHAN, J. (1955). *Brit. J. exp. Path.*, **36**, 22.

——, RAYNER, B., TUTT, M., and VAUGHAN, J. (1953). Ibid., **34**, 384.

KIDMAN, B., RAYNER, B., TUTT, M., and VAUGHAN, J. (1952). *J. Path. Bact.*, **64**, 253.

LENGEMANN, F. W., and SWANSON, E. W. (1957). *J. Dairy Sci.*, **40**, 216.

——, ——, and MONROE, R. A. (1957). Ibid., **40**, 387.

LODGE, J. R., LEWIS, R. C., REINEKE, E. P., and MCGILLIARD, L. D. (1958). Ibid., **41**, 641.

Medical Research Council, Committee on Protection against Ionizing Radiations (1959). *Brit. med. J.*, i, 967.

PALMER, R. F., THOMPSON, R. C., and KORNBERG, H. A. (1958). *Science*, **127**, 1505.

QUIMBY, E. H., WERNER, S. C., and SCHMIDT, C. (1950). *Proc. Soc. exp. Biol. (N.Y.)*, **75**, 537.

SQUIRE, H. M., MIDDLETON, L. J., SANSOM, B. F., and COID, C. R. (1957). International Conference on Radio-Isotopes in Scientific Research, Paris, Paper No. UNESCO/NS/RIC/143.

SWANSON, E. W., LENGEMANN, F. W., and MONROE, R. A. (1957). *J. Anim. Sci.*, **16**, 318.

SØRENSEN, P. H. (1956). *Årsskr. Kong. Vet. Landbohøjsk., København*, p. 64.

TUTT, M., KIDMAN, B., RAYNER, B., and VAUGHAN, J. (1952). *Brit. J. exp. Path.*, **33**, 207.

University of Tennessee (1954). USAEC Agriculture Research Programme Document No. ORO-133, p. 17.

—— (1955). USAEC Agriculture Research Programme. Document No. ORO-145, p. 85.

WASSERMAN, R. H., COMAR, C. L., NOLD, M. M., and LENGEMANN, F. W. (1957). *Am. J. Physiol.*, **189**, 91.

——, ——, and PAPADOPOULOS, D. (1957). *Science*, **126**, 1180.

——, LENGEMANN, F. W., and COMAR, C. L. (1958). *J. Dairy Sci.*, **41**, 812.

THE SPEAKER'S INTRODUCTION

The Chairman, Mr. T. A. R. Chipperfield, in introducing Mr. Garner, said that so far as he was aware Mr. Garner was the only veterinary surgeon in this country who was actively engaged in research in the radiobiological field. The work at Compton was with cattle, which was unique in the Western world. He was informed that in the U.S.A. they were working solely with pigs and sheep. Therefore, Mr. Garner was doing some very essential work and filling a gap which even the Americans were not touching.

He called on Mr. Garner to present his paper. **Mr. R. J. Garner,** in presenting his paper, said that he wished to take the opportunity to say a few words about the background to the work reported in it. The aim at Compton was to provide answers to two questions: (1) In the event of contamination of pasture with fission products to a known extent, what concentration of various isotopes was likely to be found in the milk of cows grazing those pastures? (2) At what level of contamination of pasture would attention have to be paid to possible danger to grazing animals?

Naturally enough, initially the greatest attention had had to be given to the public health aspect—the milk story—but equally naturally experiments had been devised that would also yield information on other, more general aspects, of fission product metabolism, and it was the results of those experiments in connection with two particular isotopes, ^{131}I and ^{89}Sr, that were summarised in the present paper. More recently they had been able to begin a systematic investigation of the toxicity of fission products to animals. He would have preferred to have talked to them about that side of the story which he knew would be of much greater interest to them, but the time did not yet seem opportune. However, he proposed to say just a word about ^{131}I toxicity later.

He wished to go very rapidly through one or two things in the paper. It seemed to him that the only good thing about introducing a paper was that it gave an opportunity to revise and make additions—and, in the interval between writing and presentation, he could assure them that a great many amendments and additions came to mind. But before he did this, he would like to say something of the facilities that they had at Compton for this kind of work. Mr. Garner then showed slides of the animal accommodation and equipment.

To turn to some of the results, the position with regard to ^{131}I was fairly straightforward. Some 20-40 per cent. of an ingested dose went to the thyroid gland.

The milk story was somewhat complicated, but he did not propose to go into it here.

There were, unfortunately, several errors of arithmetic in the hurried calculation of the results given for strontium and calcium. Those errors did not invalidate the arguments put forward, except in one respect which he would mention later.*

The metabolism of the strontium isotopes was perhaps confused because of the tendency always to relate strontium to calcium. Strontium-calcium inter-relationships were of greater importance in man than in farm animals since, in man, it probably true to say that any radioactive strontium which entered the body was in the form of contaminated dietary calcium. In grazing animals this was not so because fission products would usually be ingested before they were assimilated into plant tissues. However, a study of the processes involved in discrimination between the two elements provided a nice exercise in techniques. They had actually covered the 3 alkaline earth elements—calcium, strontium and barium—but he would have very little to say about the latter today.

He was quite sure that the terms "Observed Ratio" and "Discrimination Factor" were confusing to some of them. The Observed Ratio was very simply defined as the ratio of the strontium-calcium ratio in the sample under consideration (e.g., milk) to the strontium-calcium ratio in the precursor (usually the diet). Thus, if the diet contained, for every 100 parts of calcium, 100 parts of strontium and the milk contained, for every 100 parts of calcium, only 10 parts of strontium, the Observed Ratio milk-diet would be 10/100 over 100/100, or 0.1. It would be seen that if the Observed Ratio was less than unity, there must have been discrimination against strontium (or, if one liked, in favour of calcium) during their transfer from the precursor to the sample. The Observed Ratio was further defined as the product of a number of Discrimination Factors, each one

(* *The correct figures are given in this published version of the paper.*)

of which was simply a measure of the extent to which the physiological process to which it referred, e.g., urinary excretion, secretion of milk, contributed to the overall discrimination.

Those terms had originally been coined by Comar and his colleagues who had published a number of most complicated-looking formulae for the calculation of individual Discrimination Factors. He had been able to find no reference to the way in which those formulae were derived, but it seemed that it had been assumed that no discrimination occurred when strontium and calcium were secreted from the blood into the gut. This was where he wanted to introduce an amendment. In the paper he had said that the gut was apparently unable to differentiate between strontium and calcium during this secretion process, but a re-assessment of their results led them to believe that there was, in fact, some slight discrimination in favour of strontium. If this was so (and it was certainly true in the case of barium and calcium), Comar's formulae became invalid.

He had concocted some new formulae which could be shown to reduce to Comar's if the process of endogenous secretion was ignored.

The $DF_{absorptive}$, i.e., that part of the overall discrimination due to differentation during intestinal absorption, was readily defined as the ratio of the percentage of orally administered strontium absorbed to the percentage of orally administered calcium absorbed and could be obtained by comparing results after oral administration with those after intravenous administration of the 2 isotopes to the same animal. The $DF_{urinary}$ measured the effect urinary excretion had on the relative amounts of strontium and calcium remaining in the body and, similarly, the DF_{faecal} represented the effect of secretion from the blood into the gut. The $DF_{lactational\text{-}milk}$ was a measure of the effect the udder had in discriminating between the amounts of strontium and calcium which had appeared in the milk. The $DF_{lactational\text{-}body}$ represented the effect which the loss of strontium and calcium in the milk had on the amount remaining in the body. These Discrimination Factors were obtained from the appropriate Observed Ratios (which could, of course, be measured) by making use of the fact that the Observed Ratio was defined as the product of all the Discrimination Factors involved.

It would be seen that the major physiological processes responsible for the discrimination between strontium and calcium (and for that matter, between barium and calcium) during their passage from blood to milk were intestal absorption and mammary secretion. Despite the fact that the kidneys could discriminate very efficiently between strontium and calcium, as shown by the high $OR_{urine\text{-}injection}$ following i/v administration, so little strontium and calcium were execreted via this route that their effect on overall discrimination was small—perhaps this illustrated the value of the Discrimination Factor.

He had mentioned in the paper that direct estimates of the toxicity of ^{131}I to cows were being made. They might be interested to see what a cow with, as far as they could tell, its thyroid gland completely destroyel by ^{131}I looked like.

Mr. Garner then showed slides of a cow which had received 140 mc. ^{131}I over a period of 14 days in June last year; she had been photographed in July last when she was about 8 months pregnant. He size was not entirely due to her pregnancy—her girth when 3 months pregnant had been over 9 feet as compared with 7 feet or so in control animals. Another change, besides putting on weight, had been thickening of the legs; this had more less disappeared in the hind legs but was still apparent in the fore legs. There had also been a scurfiness and thickening of the skin over the neck, harshness of the coat and, above all, loss of interest in anything but eating.

The Opener

The Chairman announced that Mr. G. B. S. Heath, who was to have opened the discussion, was unwell, and that Mr. Lloyd would deputise for him. He was sure that the meeting would like to send to Mr. Heath good wishes for an early recovery.

Mr. Lloyd was in charge of the new Isotype Laboratory at Weybridge, where they intended to undertake countrywide monitoring of strontium and iodine in the bones and thyroid of sheep, which was part of the national programme. Mr. Lloyd had spent a year at the Oak Ridge Establishment in Tennessee, and he felt sure that he would be an able deputy for Mr. Heath.

Mr. M. K. Lloyd, in opening the discussion, said that he regretted that Mr. Heath was not able to address the meeting, for, as many of them would know, Mr. Heath was a capable speaker, and furthermore, having been directly involved in the Windscale accident, as far as its effects on animal health were concerned, he could have made a more balanced appraisal of the importance to the profession of Mr. Garner's work than he (Mr. Lloyd) would be able to advance.

Not many years ago, when he had been a student at Liverpool, Mr. Garner had been his lecturer in biochemistry and as such at the end of the year had no doubt had to open the discussion on his (Mr. Lloyd's) year's work before the board of examiners. Today there was an interesting reversal of rôles, for he was asked to open the discussion on Mr. Garner's work. He took it that at that earlier meeting Mr. Garner had dealt kindly with him, for he had been permitted to leave the biochemistry class to proceed to his clinical studies.

His remarks today would not depend on any practical experience of the topic, for other than at Compton and the University of Tennessee Agricultural Research Program at Oak Ridge, very little experimental work on these lines using cattle had been carried out. They might judge the results from the fact that there was good agreement in the data from both institutions. The paper spoke for itself, and he thought it would be agreed that Mr. Garner was to be congratulated on putting before them a thorough account in a simple and readily comprehensible form, from which they could get a good working idea of what would happen in the cow, and to its products, in the event of further increases of fall-out, or accidental discharges from nuclear plant.

He thought it was worth drawing attention to the technical difficulties which must have beset Mr. Garner in this work. The isotopes he had used were regarded as some of the more hazardous to handle. Large doeses, compared to usual biological tracer work, were required, for the dilution factor in the cow was great. The end result was an unpleasantly large quantity of radioactive faeces, urine and milk which had to be handled, sampled and disposed of without allowing spread of contamination. Afterwards there was still a radioactive cow to contend with. She would no doubt stand there looking rather unconcerned and from time to time produce a radioactive belch. So that while the principle of the method might seem simple, a good deal of care and thought in the design of the metabolism unit, and the experimental procedure, was necessary in order to avoid risk.

In spite of those difficulties, it would be agreed that it was very necessary to make these studies on cattle rather than to make indirect assessments or extrapolate the results from laboratory animal experiments. This could be appreciated from Mr. Garner's statement that the rates of I^{131} uptake by, and loss from, the thyroid of cattle and sheep were different from those in most other species.

Mr. Garner had pointed out that, from a consideration of the available data, if a cow's milk was sufficiently low in I^{131} and Sr^{90} as to be fit for human consumption, then there was no danger to the cow itself. It therefore appeared reasonable that these studies should be discussed from the point of view of milk inspection and public health. Milk inspection was a study of considerable concern to the profession and it was therefore important that they should be fully alive to the information in Mr. Garner's paper, and its significance. This new milkborne hazard had already become a reality, for as they would remember at the time of the Windscale incident milk produced in that area had been withdrawn from sale because of the possible hazard. Although the methods used in detecting hazardous levels of these fission products were not particularly simple or readily lend themselves to "on the spot use", one could acquaint oneself with the likely consequences of what one might term a "nuclear situation" and make some assessment of what action should be taken until analyses demonstrated

positively whether or not a given milk sample was safe.

The Windscale incident was worth considering further in connection with this paper. Measurements made by the Radiological Protection Service at that time as far away as the London area had shown levels in the thyroids of persons tested of up to 6 mμC I^{131} falling to around 0.1 mμC 80 days after It was almost certain that this activity derived almost entirely from cow's milk. I^{131} had been detected in London milk during the period but the levels had been too low to be stated with confidence. Measurements made at Weybridge on thyroids from sheep grazing the Whitehaven area at the time had risen as high as 7 μC I^{131} in one group, and at the same time sheep in the North of Scotland had shown levels of 0.01 μC. The levels in the Whitehaven sheep had declined exponentially to 0.1 mμC in late January—a little over 3 months after the escape. For comparative purposes the maximum permissible total body burden of I^{131} was 0.6 μC.

I^{131} in milk need not be such a great problem, provided its presence was detected and the level known, for contaminated milk could be dried. made into butter or cheese or otherwise processed to a non-perishable form, and stored to permit the radioactivity to decay. The level would, for instance, decline a thousandfold in 80 days.

Mr. Garner had drawn attention to the uniqueness of the grazing animal in being able to collect the debris from a considerable volume of air and concentrate this in its various products, particularly milk. One might add that the speed with which certain of this debris was transferred to the milk jug on the breakfast table was also unique and of special importance in the case of I^{131} with its fairly short radioactive half-life of 8 days, and its volatile nature.

This question of the time factor was of some interest because of the effect of differing half-life in the fission products deposited. Considering only the principal biologically significant isotopes resulting from slow neutron fission of U^{235}, one day after fission the short-lived isotopes I^{133}, I^{135} and Sr^{91} predominated; after 20 days it was Ba^{140}, I^{131} and Sr^{89} which were present in greatest concentration. After 1 year Sr^{89} and Sr^{90} and after 20 years Sr^{90} and Cs^{137}. In considering this list it was, of course, important to remember that the total activity of the fission producct was declining. From an examination of the list it could be seen that strontium entering the food chain to be metabolised in the cow would show a higher ratio Sr^{89}/Sr^{90} if the source was more recent fission; this was in fact observed, for the ratio in animal and human bone was higher shortly following release of fission products from a bomb test, and subsequently declined.

Furthermore, in a nation-wide average cow's milk sample the Sr^{89}/Sr^{90} ratio was found to fall in winter to a lowest level in March, and this was due to the fact that the feed of cattle in winter had been stored for some time, thus permitting the Sr^{89} activity with its shorter 54-day half-life to decay out relative to Sr^{90} which had a 28-year half-life.

It had also been possible using this ratio to show that Sr^{90} in the milk of grazing cows was largely due to fallout deposited during the previous one or two months. This was an important fact, for it meant that levels in milk, the principal source of Sr^{90} in human diet, were controlled more by recent deposition than by the cumulative deposition.

Mr. Garner's paper included a list of metabolic discrimination factors against Sr relative to Ca, derived from tracer experiments. Stable strontium was present in trivial quantities in the diet of cows and humans, and was so far not known to have any metabolic significance. In the case of humans the ratio of this stable strontium to stable calcium in the average diet compared to that in the bones had been used to determine the discrimination factor; it had been found to be 4. If a similar estimate of the average cow diet could be made it might be useful to apply the method to the D.F. diet to milk in the cow and compare the result with the isotope method result.

The secretion of Sr^{90} from blood to milk was an important feature, and it was interesting to note, as Mr. Garner had pointed out, that secretion of Sr^{90} was related to milk yield, whereas I^{131} secretion appeared to be independent of yield. The University of Tennessee team at Oak Ridge had observed that after oral and intravenous doses of Ca^{45}, the specific activity, i.e., the ratio of Ca^{45}/Stable Ca, had been higher in milk than in plasma of cows. This observation had appeared at first sight theoretically impossible, and several factors which might have accounted for it had been investigated; it had been concluded that the explanation was the existence of a calcium store in the udder, where calcium was held and was not exchangeable with calcium in the plasma. It would be interesting to learn whether a similar phenomenon would be observed in the case of the ratio Sr^{90}/Stable Sr or Sr^{90}/Stable Ca, and whether there was any storage of strontium in the mammary tissue as opposed to a direct secretion from plasma to milk.

Very little information on the mechanism of passage of calcium across biological membranes was available, and this would seem pertinent to a more detailed consideration of the discrimination between Ca and Sr in the body.

The 1958 levels of Sr^{90} in cow's milk in this country showed a mean value of approximately μμC/gm Ca; there were considerable variations from season to season and from area to area. In some districts, especially the hilly areas with high rainfall, the level might be as high as 30 μμC/gm Ca. The United Nations Report indicated a world value of just over 1 μμC/gm Ca in 1954 and the levels had steadily increased since then. Applying the discrimination factor given by Wassermann and his co-authors, this indicated in 1958 a mean level of 70 μμC/gm Ca in the diet of cattle.

Dr. Janet Vaughan had drawn attention to another interesting feature of Sr^{90} metabolism which was not shared by stable strontium. On decaying Sr^{90} became yttrium^{90} which was also radioactive with a half-life of 64 hours and a harder β radiation. The radiation dose due to Sr^{90} in bone delivered to bone and more particularly to bone marrow was due mainly to Y^{90}. Yttrium differed chemically from Sr and Ca, and so did its behaviour in the body. However, in the case of bone this did not make much difference as the Y^{90} produced on decay of Sr^{90} remained firmly in the bone. In the retina of the rabbit Sr^{90} was concentrated to a greater extent than in other soft tissue. The Sr^{90} could pass in and out of the retinal tissue but the Y^{90} produced there did not appear to pass out and thus the retina retained even more Y^{90} and Sr^{90}. It was also found that yttrium could not enter the retinal tissue. Freak aspects of metabolism of isotopes which decayed to active daughters such as this might be of some significance in assessing hazards and doses to various tissues.

He hoped that these few remarks would not have been too indigestible. The topic of fission products did not, as they would appreciate, readily lend itself to a light or flippant discussion. And in closing he would like to say that he hoped for all their sakes that they never need make use of this information on fission product metabolism in real earnest.

The Reply

Mr. R. J. Garner, in replying to the discussion, said that the answer to Mr. Calderbank was that no calves had been born yet. The plan of the experiment was that 4 cows were treated at each dose level, of which there were 6. They slaughtered one cow from each dose level and retained the other three for observation. The fat one that he had shown in a slide was the first one due to calf, and the other two cows on that dose level were in calf.

He had deliberately kept off the subject of I^{131} in milk, because he had given a paper on that aspect to the Gleneagles Conference in April which he had hoped would have been published in *The Veterinary Record* before the present Congress, but unfortunately its publication had been delayed by the printing strike. The amount of iodine appearing in the milk was now generally recognised as being directly related to the amount consumed with the diet. It was one of the only things, so far as he knew, whose level in the milk was directly connected with the level in the diet.

With regard to active secretion, iodine was definitely actively concentrated by the mammary gland. They had convinced themselves by demonstration of the presence of an iodine trap similar to that in the thyroid gland. Presumably the mammary gland did compete with the thyroid for iodine, although he would have thought that it was a pretty unsuccessful competitor.

In reply to Mr. Holman, one got the same discrimination

* mμC=millimicrocurie. mC=millicurie. μC=microcurie.

factors and observed ratios with different levels of strontium. They had used doses varying by a factor of 10 and had got the same answer. He thought that if one increased the dose very much and got pathological changes, one certainly would not get the same answer, but otherwise one would be pretty safe in assuming that one would get it.

So far as he knew, there was no other species in which the thyroid gland had been destroyed with such a small effect—not with no effect, but with such small effect. On the question of fertility, they had not been so much afraid of thyroid destruction having an effect on fertility as of the whole body irradiation having an effect on fertility.

In reply to Dr. Phillipson, there was a thing called the relative biological efficiency which was always taken into account when calculating the radiation dose—the absorbed dose which was measured—which was given by any isotope to a tissue, but in the case of Beta and Gamma radiation it was usually assumed that the relative biological efficiency was the same.

It was usually assumed that the Beta radiation was the more dangerous of the two, because radiation from it was absorbed in a much smaller mass of tissue and therefore the effect was more intense. With iodine which had both Beta and Gamma radiation one usually measured the Beta dose and based things on that, and that was what they had done. In fact, although they had talked about a dose of 100,000 rads, this was a bit "in the air", because it could have been 200,000 rads; the vital thing was that they knew how much had to be eaten by the cow before the thyroid was destroyed. If they did not know that, they would have to work backwards from a radiation dose and calculate what would be likely to give that radiation dose. (Incidentally, a "rad" was that amount of radiation which led to absorption by the tissue of 100 ergs of energy per gramme of tissue, an erg being simply a measure of energy on the C.G.S. scale.)

In reply to Mr. Goodwin, official monitoring over the whole country had been put in the hands of the Agricultural Research Council last year or the year before, and reports were being published every 6 months, the first one having come out at the end of July. This monitoring covered animal bones, milk and anything which people were likely to eat, including cabbages, potatoes and other vegetables, bread, and so on. The whole monitoring programme was in the hands of the Agricultural Research Council. This had come about simply because it had been felt that monitoring, which had hitherto been in the hands of the Atomic Energy Authority, should come under the aegis of someone not directly interested. As to the question about whose responsibility it was to check milk levels and who made decisions on the public health side, he was afraid that he did not know who made the decisions, but he assumed that at Windscale it had been the Ministry of Agriculture, Fisheries and Food. The actual measurements were done by the A.R.C. He believed that the decision to dump milk after Windscale had been a joint one by the Medical Research Council and the Ministry of Agriculture, Fisheries and Food.

There were several points in Mr. Lloyd's opening remarks to which he wished to refer. Mr. Lloyd had mentioned the amount of iodine in the thyroids of sheep after the Windscale incident. Dr. Holman had already answered the point made in that connection. In the human there were so many safety factors that were taken into account. First, one said that the lowest level likely to cause any damage was so-and-so, and then one said that there might be circumstances where the amount that might produce damage would be 10 times less, and then one said that another safety factor of 10 should be included anyway—so that one ended with a quantity which was called the maximum permissible level and was one which under any circumstances would not cause any appreciable change whatsoever. That was not a thing in which they were interested at Compton in the case of animals; they were trying to work out what they called the minimum effective doses, and the 7μc in a sheep would have to be exceeded by at least 25 times in a sheep to produce any pathological changes in the thyroid gland.

Mr. Lloyd had also mentioned the importance of using stable strontium as an indicator of strontium metabolism. They were working on this. As Mr. Lloyd had said, there was very little stable strontium in grass; it was a rather difficult thing to measure, but they had acquired the equipment and were engaged in trying to develop good methods for estimating stable strontium in grass, cattle cake, blood, milk, and so on. In fact, they intended to use the stable strontium and calcium in the food as a marker for comparing continuous ingestion with a single dose.

Mr. Lloyd had also mentioned the possibility of there being a strontium store in the udder similar to the calcium store which was believed to exist. He was himself convinced that there was a strontium store in the udder; there were pointers to this, but there was no direct evidence.

Finally, on the question of the metabolic dissociation between strontium 90 and yttrium 90, in a cow this dissociation was not going to be of great importance, because there was so much more strontium that went to the bones than to the eyes. But with Barium 140 the isotope behaved rather unlike its sister elements, calcium and strontium, in that it was taken up to a phenomenal extent by the eye. In the cow the concentration in the choroid on a wet weight basis could be 10, 15 or 20 times greater than the concentration in the bones. Therefore, with this particular isotope the choroid might be the critical organ, although he did not think that people would have to worry too much, because before one got radiation damage to the choroid the milk would be completely unfit for human consumption.

The Chairman said that his interest in this subject had first been stimulated by his representing the Sussex Veterinary Society at the study course at Sunningdale, where he had arrived with the feeling that, of course, this was a catastrophic subject, that if a hydrogen bomb fell on this country it would be such a catastrophe that there would be nothing that could be done about it. But the more one learned about the subject in all its aspects, the more one became aware of the size of the problem, the more one realised that there were means of dealing with it, the more intrigued one became. For one realised that we were standing on the very fringe of the unknown.

Even at his time of life, he would be happy, if he had the opportunity, to undertake the study of this subject from the very beginning. Flowing from that, he knew that the veterinary schools had a very overcrowded syllabus, but he hoped that they would be able to incorporate in their syllabus a certain amount of time for teaching the elements of radiobiology so that the students might be stimulated with the spirit of adventure to go in and represent the profession in this field. They had very few men who were either qualified or willing to do this work. He would like to see young veterinary students going out from the colleges and undertaking this work. It was not just a question of civil defence. They were on the fringe of something which was exciting and which was going to lead to things as yet undreamed of.

He would like to see post-graduate courses either in the veterinary schools or a Compton or in the universities where graduates could go and possibly obtain a degree in radiobiology. He also pleaded for the rapid dissemination of newly acquired knowledge. One of the things that the profession had lamented in the last few years was that they had known that a lot of things were going on, but they had known so very little about them.

In the near future there would be the question of the practical application of isotopes not only for research but possibly for clinical purposes. One example was the husk vaccine, which was a commercial application of modern research.

There was one other point to which he wished to refer and which he had raised with the leader of the group at the study course at Sunningdale. It was the legal position of owners who might fear that their stock had been injured or in some way damaged by accidental escape of radioactive material. He had foreseen a situation in which the uninformed practitioner would be called upon to advise a client. He had, however, been reassured by the leader of the group at Sunningdale, who had told him that the position would shortly be covered by very comprehensive legislation; that the proprietors of atomic stations, whether commercial such as the atomic power stations, or research places such as Harwell, would be made absolutely liable for any damage that might flow from the escape of radioactive material from those premises. He had gathered, although the legis-

lation had not yet seen the light of day, that in drafting it there would be a clause whereby a claim might be back-dated even 30 years.

He had stressed that they knew so little about this subject and that what the authorities were interested in was getting at the truth. When an accident like that at Windscale occurred, the procedure from the client's point of view was that the Ministry of Agriculture and Fisheries alerted the Medical Research Council and the Agricultural Research Council, and from those bodies independent experts went to assess and evaluate the damage to the individual. When that had been done, the proprietors of the plant were liable. This, he thought, put the position of practising veterinary surgeons in a rather different light; he had been a little fearful that they might get involved in matters of which they had little understanding and find themselves out of their depth.

Having made those remarks, he thanked the author of the paper, the opener of the discussion, and all those who had taken part in the discussion.

Broiler Diseases

BY
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SUMMARY—Accurate information on the economic loss from disease in broiler plants in Great Britain is not available, but evidence would suggest that the disease position compares favourably with that reported in the United States.

On the other hand, a number of the diseases present in the broiler areas of the U.S.A., to which heavy losses have been attributed, are now occurring in this country. Although it is as yet difficult to assess their importance economically, the potential hazards resulting from intensivism accompanied by an increase in unit size in broiler flocks must be borne in mind. The margin of profit in broiler production is such that, apart from acute infection with high mortality, any interference in growth, food conversion and carcase quality can have serious economic repercussions for the producer.

The diseases which have appeared in broiler plants in Great Britain in recent years, and which are described in this paper are: pullorum arthritis, E. coli septicaemia, salmonellosis, epidemic tremors, infectious synovitis, chronic respiratory disease and infectious bronchitis, coccidiosis, blackhead, haemorrhagic syndrome.

It is of interest to note that fowl paralysis is occurring at an increasingly early age and in some broiler flocks reaches epidemic proportions.

The paper also discusses the prophylaxis, therapy and control of these diseases.

IT is usual, in surveying the causes of mortality in any species of livestock, to attempt to hazard some estimate as to the resulting economic loss and the relative importance of the diseases concerned. Although this is possible in the case of laying poultry, in that reasonably accurate estimates can be made from the analyses of laying test records, no such factual information exists at present with regard to the broiler industry in this country.

The annual reports of the Council of American Poultry Tests show that at various broiler trials the average mortality varied from 4·3 per cent. at the Georgian Broiler Test to as high as 11 per cent. at the Maine Broiler Test, the mortality in different entrants' stock ranging from as low as 1 per cent. to as high as 30 per cent.

Coles (1959), however, reports that from a list of broiler crop costings in England and Wales examined by him over the last two years, and which relate to a total of 1,368,997 birds, the average mortality rate was 3.97 per cent. He also states that summaries from other groups amounting to approximately 5/6 million birds, whilst less reliable, still indicate a surprisingly low average mortality rate of between 3 and 6 per cent.

At present, therefore, it would appear that mortality is probably lower in this country, and certain diseases, particularly the respiratory infections, have not yet reached the high levels stated to occur in the more densely populated broiler areas of the States. Nevertheless, one must bear in mind the potential hazards which may arise from the expansion in intensivism accompanied by an increase in unit size for broiler plants. The margin of profit in broiler production is such that, apart from acute infection with high mortality, any interference in growth, food conversion and carcase quality can have serious economic repercussions for the producer. Temperton (1953), in discussing the economics of table poultry production, stated that a mortality higher than 10 per cent. could exert a more serious effect on profitability than a £10 per ton increase in food costs, whilst Golden (1953) suggested that a 3 per cent. mortality plus 4 per cent. culls has approximately the same effect as a £5 per ton increase in food costs.

Reid (1959) states that in the United States the average broiler mortality is approximately 5 per cent. and that on last year's production of 1,450 million broilers the loss was 72½ million. This mortality cost the producer $13½ per 1,000 broilers—or taking mortality, lower performance and the cost of medication and vaccines together, a total of $47 per 1,000 broilers. Smith (1959) states that in Delaware mortality in broilers was 6·5 per cent. for 1957 and the cost of mortality plus treatment amounted to $56.9 per thousand broilers. The total loss from disease in the State of Delaware was approximately $7,000,000.

In an attempt to ascertain the relative importance of the various diseases concerned, an analysis has been made of material submitted to the routine diagnosis service of the Houghton Poultry Research Station. Unfortunately, adequate histories have not always been available so that it has been impossible to differentiate stock from broiler plants from those received from other rearing farms. In Table I, therefore, the diseases listed are those encountered in all chicks received which fall in the age-group 0/8 weeks. For comparison, this table also shows the mortality experienced in the diagnostic laboratories of Delaware, North Carolina and Virginia. (Extracted from *Avian Diseases*, 1958.) Unfortunately, these data again do not differentiate between chicks from broiler plants and those from other types of farms. However, Levine (1959) states that, although the incidence of various diseases is broken down according to the age of birds submitted, so that the incidence cited in this table could well belong to replacement birds in addition to broilers, he thinks it would be safe to consider that the great majority of the cases submitted in the States of Delaware, North Carolina and Virginia would represent conditions found in broilers, since the poultry production in these three States is predominantly broiler production. It would be dangerous to place too much reliance on these figures as an indication of the relative economic importance of the

TABLE I
MORTALITY IN GROWING STOCK, 1957

	H.P.R.S.	Delaware	N. Carolina	Virginia
	0–8 weeks		0–5 months	
Respiratory diseases*				
p.p.l.o.		696	155	255
I.L.T.		117	—	52
I.B.	377	20	20	54
Coryza		7	7	7
Fowl Pox		11	11	21
Newcastle disease†	—	201	4	29
Omphalitis	119	49	16	8
Salmonellosis	82	5	19	14
Coccidiosis	49	86	138	255
Pullorum	46	8	8	49
Epidemic tremors	39	57	44	67
Encephalomalacia	23	1	1	7
Fowl paralysis	19	176	‡	
Leucosis (incl. visc. lymphomatosis)			61	359
Haemorrhagic syndrome	11	74	62	11
Perosis	8	6	—	—
Aspergillosis	8	1	15	19
Blackhead	3	21	17	61
Rickets	1	—	19	25
Avitaminosis A	1	1	31	3
Ascariasis	—	32	148	77
Non. Spec. Enteritis	19	103	163	58
Infectious synovitis	—	146	4	144
Capillaria	—	—	66	4
Undiagnosed or unclassified	226	—	—	—

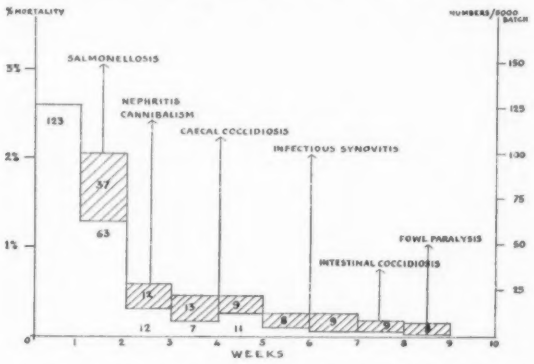
*Undifferentiated in the case of H.P.R.S.
†Newcastle sediase records not included in H.P.R.S. data.
‡Undifferentiated

various diseases encountered, since in most instances the histories available are inadequate for the purpose of assessing the individual losses experienced in any particular outbreak.

So far as the Houghton records are concerned, it should be noted that no effort has been made to differentiate between the various types of respiratory diseases, since when the records were compiled material was being submitted to other institutes investigating these conditions. Furthermore, no records have been kept of outbreaks of Newcastle disease which were reported to, and dealt with by, the Animal Health Division of the Ministry. The fact that no infestations with *Ascaridia* or *Capillaria* were recorded at Houghton may be due to the fact that our records deal with chicks up to 8 weeks of age only, whilst the American figures may include birds up to 5 months. Similarly, although no outbreaks of infectious synovitis were recorded at Houghton for 1957, this disease has since been reported in this country.

Blount (1959) illustrates in chart form the times at which disease exercises its main effects on broilers, and this is reproduced as Table II. This shows that during a 70-day growing period when losses are at the normal level of less than 5 per cent. the highest number of deaths occurs during the first 10 days of life. If, however, there is any actual outbreak of disease, secondary peaks will follow as indicated. Initially, a mortality of 2–2½ per cent. is normal during the first week, followed by half that amount the following week. This means that in a batch of 5,000 broilers, from 100 to 125 chicks may die during the first fortnight out of a total of 250 deaths for the full 70-day period. If double figures are exceeded on any day after the first 10 days, action should be taken.

TABLE II.—Graphic Representation of the Normal Mortality Picture Amongst Broilers to 10 Weeks of Age.



In view of the limitations of the data already mentioned, it would be unwise to deal with these diseases in the order of their occurrence since this might result in an undue prominence being given to some diseases at the expense of others. The best that can be done, therefore, is to comment on new diseases which have appeared in broiler plants in recent years and to review developments which have occurred in the control and treatment of such diseases. In order to approach the matter in a logical sequence, the diseases are dealt with according to their aetiology, i.e., bacterial, viral, protozoal, environmental; this order does not in any way indicate their relative importance or priority.

1. Bacterial

(a) Salmonella Infections

Although outbreaks of salmonellosis in poultry are well known, there are one or two recent findings of some interest, particularly in relation to broiler production. A recent paper by Carnaghan and Sojka (1958) described three outbreaks of arthritis in broiler chicks in which a variant strain of *S. pullorum* was incriminated. Average mortality varied around 5 per cent., although in one outbreak some 200 deaths occurred in a 1,000-bird unit. The predominant symptom was lameness, accompanied by marked swelling of the hock and foot joints, poor feathering and underdevelopment. Deaths mainly occurred between 10 days and 5 weeks of age. On autopsy an orange-coloured gelatinous exudate was found in the joints, whilst necrotic lesions were found also in the myocardium and occasionally in the liver. In two of the outbreaks the chicks originated from the same supply farm.

The disease could be reproduced experimentally both by inoculating a crude emulsion of the joint exudate and by feeding a broth culture of the *S. pullorum* variant isolated from the lesions. Survivors of the experimentally produced infection did not react to a blood test carried out with standard pullorum

antigen, but reacted positively to an antigen prepared from the variant strain. These findings suggest that the disease had been transmitted, the carrier birds having escaped detection at the original blood test.

Variant strains of pullorum are known to occur in this country, but their occurrence does not appear to be of significance in relation to routine blood testing (Blaxland, 1954).

Although no therapeutic controls were reported, it would appear probable that such outbreaks might respond to the use of furazolidone in the food at the 0.04 per cent. level.

Outbreaks of disease caused by members of the *Salmonella* group other than pullorum and gallinarum are by no means uncommon in broiler plants. In fact, it will be noted from Table I that 82 outbreaks were encountered during the 12-month period covered. Although the majority of outbreaks in this country are caused by *S. thompson* and *S. typhimurium*, outbreaks from which rarer species of *Salmonellae* can be isolated are occasionally encountered. A total of some 50 *Salmonella* species have now been isolated from poultry in this country, and during the last year we have isolated a number of species not previously reported in chickens in Great Britain, i.e., *braenderup*, *muenchen* and *orion*. Blaxland, Sojka and Smither (1958) have reported the isolation of *S. mbandaka*, *S. weybridge* and *S. newhaw*. Fortunately, these exotic species do not tend to become established in our poultry flocks and the outbreaks usually tend to be restricted to the group concerned; in many instances the mortality rate may be extremely low.

It is interesting to speculate whether there is any association between the occurrence of these exotic types and the facts recently reported by Walker (1957) and the Ministry of Health and Public Health Laboratory Service (1959), which indicate that *Salmonella* organisms are present in many of the raw materials used in fertilisers and animal foodstuffs and also in the finished products. Of 1,262 samples examined, 24 per cent. yielded *Salmonellae*, the most heavily infected substances being bone and meat meal and marine products. Of 71 samples of complete feedingstuffs examined, 8 per cent. yielded *Salmonellae* and 14 out of 17 samples taken from the environment of factories were found to be infected.

It is not yet possible to assess the importance of *Salmonella* organisms in animal feedingstuffs and further work is required into their epidemiological significance. It seems probable that the *Salmonellae* are present in relatively small numbers in contaminated material, although multiplication may occur in mashes under suitable conditions of moisture and temperature.

Numerous workers (Smith, 1955, and Wilson, 1955) have shown that furazolidone at 0.04 per cent. in the diet effectively controlled mortality resulting from *S. typhimurium* experimental infection. Unfortunately, however, a high proportion of the treated chicks remain carriers, although they appear healthy and grow normally. Obviously it would be unwise to retain such treated chicks for breeding purposes, and from the public health viewpoint a high proportion of carriers following on treatment might be of some consequence, especially in view of the excretion of resistant strains of *Salmonellae* following on the feeding of antibiotics in broiler rations, which will be referred to later.

(b) *E. coli* Infections

The pathogenicity of organisms of this group for the chicken has not until recently been well established, although coliforms primarily occur as secondary invaders in C.R.D. and as a cause of mortality in baby chicks. Recently, however, a number of outbreaks in broiler chicks of about 6–10 weeks of age, in which *E. coli* could be isolated in pure culture from various organs and would appear to be a primary cause of mortality, have been encountered.

Similar outbreaks have been reported by workers at the Central Veterinary Laboratories, Weybridge (Sojka, and Carnaghan, 1959). These workers report that certain serological types of *E. coli* isolated from cases of coli septicaemia are capable of reproducing the lesions seen in the field outbreaks of the disease when administered by various routes. In many cases *E. coli* was the only pathogen involved.

In the outbreaks observed both at Weybridge and Houghton it has been difficult to asses the morbidity, but since there was a fall in food intake the number of carcases downgraded was frequently higher than normal. The mortality rate has varied from 7 to 10 per cent. Weybridge workers report that the disease is commoner during the winter months, and therefore may be associated with faulty ventilation, particularly in those instances where windows or air inlets have been closed in order to maintain a high temperature within the house; other experiments indicate that the disease may be stepped up by inhalation, a method of spread which can be highly important in view of the concentration of susceptible birds in broiler houses.

Symptomatically, the disease shows some resemblance to C.R.D. in that there is usually sneezing and respiratory distress but an absence of nasal discharge or facial swelling. The most constant finding at autopsy is extensive fibrinous pericarditis, accompanied by a gelatinous exudate covering the surface of the liver, and an accumulation of yellow caseous material in the abdominal and thoracic air sacs. These air sac lesions may also result in the rejection of the carcase at the packing station.

At Weybridge, sensitivity tests have shown that all isolates in field outbreaks have been susceptible to furazolidone, and resistant to the tetracyclines. Similarly, at Houghton, a number of cultures tested have also proved resistant to the tetracyclines. At present, therefore, a course of furazolidone at 0·04 per cent. in the food over a period of a week to 10 days has been recommended, with promising results.

It is interesting in this respect to note that Barr and Carman (1957) described an outbreak of fatal diarrhoea in a flock of 3,000 ten-week-old broilers in which the causal agent was considered to be *E. coli*. The flock was on a ration containing tetracyclines, and the organism isolated was resistant to both chlortetracycline and oxytetracycline but responded to a combination of neomycin and polymixin. These authors consider that the number of tetracycline-resistant strains of *E. coli* is on the increase as a result of the continuous use of these antibiotics in the food.

Workers at Houghton (Gordon, Garside and Tucker, 1959) have shown that when chicks were fed chlortetracycline at 25 gm./ton continuously from day-old the percentage of chicks excreting resistant coliforms varied from 40 to 70 per cent. When chicks received the drug at the high level of 400 gm./ton intermittently, 98 per cent. excreted resistant coliforms. This figure, however, gradually fell to about 10 per cent. 8 weeks after the antibiotic was discontinued.

Work in progress has also shown that when chicks receiving 100 gm./ton chlortetracycline in the ration were experimentally infected with a strain of *Salmonella typhimurium* sensitive to chlortetracycline at 25 p.p.m., a ninefold increase in resistance occurred stepwise by the third passage, to 225 p.p.m. This work indicated that these resistant strains stemmed from the challenge organism and not from resistant strains already present.

The emergence of *Salmonellae* resistant to antibiotics may be of importance from the public health standpoint (Barnes, 1957; Shrimpton, Barnes and Miller, 1958), particularly in relation to the use of antibiotics in food preservation and in view of the increase in the number of serotypes isolated from poultry in Great Britain, already referred to.

(c) Omphalitis

The death of embryos and chicks resulting from the infection of the yolk by a number of bacteria frequently present in the alimentary tract and on the skin of the hen was reported by Harry in 1957. The same author reported outbreaks in which *Staph. aureus* of human origin appeared to be the cause of the yolk infection.

Such yolk infections in chicks are usually initiated by bacteria relatively non-pathogenic in sites other than the yolk, and which possess enzymes capable of degrading the yolk protein complex, or by toxigenic bacteria capable of stimulating the production of an inflammatory exudate in the yolk sac lining. Factors which contribute to fatal infections of this type are a low standard of hygiene on the supply farm, tending to increase the contamination of the eggs with bacteria capable of setting up such infections, together with sub-optimal conditions of incubation and brooding of chicks, which delay yolk absorption and lower resistance.

The disease occurs in two forms, either as "mushy chick" disease in which losses occur at any period up to 10 days after hatching and which is characterised by an inflammation of the yolk sac, denaturing of the yolk, and transformation of its normal greenish-yellow translucent emulsion to a brownish turbid fluid or to a solid yellow mass, or as true omphalitis or "navel ill", which is characterised by necrosis and oedema of the tissues in the proximity of the navel.

In two outbreaks of yolk sac infection with *Staph. aureus* which involved both embryonic and chick mortality, the causative organism was traced to the persons handling the eggs on the eighteenth day and they were found to be either nasal or skin carriers of this organism. The use of barrier creams by hatchery operatives would be an advantage, whilst other control measures could be effected by improved hygiene on both the supply farm and the hatchery. Fumigation methods, aerosols and the germicidal dipping of hatching eggs are referred to by Gordon (1956). Other factors which assist in the control of these infections are the proper storage of eggs prior to hatching, the removal of hatched chicks from the incubator as soon as possible after they have dried, gentle handling of chicks particularly during sexing, and the storage of chicks in a well-ventilated room prior to their despatch.

2. Viral

(a) Infectious Avian Encephalomyelitis

This disease, also known as epidemic tremors, was first encountered in this country about 1951, although the first report by Markson and Blaxland did not appear until 1955. Since the spring of 1957, both the incidence and the severity of the disease has increased, and it would now appear to be fairly widespread in Great Britain and a serious cause of chick mortality. The same authors (1958) recorded over 100 outbreaks during the first 11 months of 1958. Although chicks are susceptible up to 6 weeks of age, the majority of outbreaks encountered have occurred in chicks between 2 and 4 weeks of age and in some cases as young as one week. There has been a suggestion in some instances that chicks dying on the hatching trays, or during transit, may also have been affected with this disease. The disease occurs in most of the common breeds and crosses. Symptoms, although fairly characteristic, may be difficult to differentiate clinically from other nervous conditions, and in most instances it may be wise to report suspected outbreaks in the first instance as fowl pest so that the possibility of this disease can be eliminated.

In the early stages of the disease, affected chicks become unsteady on their legs and show a tendency to flop on their hocks. This is followed by staggering and ultimately by complete paralysis, the chicks falling on their sides or struggling to balance themselves with their wings. Fine, rapid tremors may be seen in the head and neck or may be felt if the chick is lightly held. Death follows in a few days, usually from starvation or as a result of suffocation or trampling. In most outbreaks the symptoms cease by the sixth week of age. Outbreaks are spasmodic in occurrence and seldom conform to a regular pattern. Losses may appear in a group of chicks from the same hatch and extend over two or three consecutive hatches, yet no losses may be experienced in subsequent hatches even when the stock, incubation, hatching conditions, etc. remain the same. There is no real evidence of any spread of infection from one group of chicks to another or from one outbreak to the next. The manner by which the disease spreads is uncertain. Experimental work strongly suggests that infection is egg-borne, but so far all efforts to transmit the disease by natural means have proved ineffective. If egg transmission occurs, presumably adult fowls must act as carriers. This is possible, since in all outbreaks there is a percentage of chicks which do not show symptoms and some survivors may develop normally. It is thought that such survivors eliminate the virus via the reproductive tract and in a few outbreaks the disease has been preceded by a reported decrease in

hatchability. Despite this evidence suggesting egg transmission, it has been difficult in most instances to suggest a possible origin of infection in the breeding stock concerned, and in some cases chicks from the same hatch, retained by the breeder, have shown no evidence of the disease. It is possible, therefore, that environmental factors such as overcrowding or nutrition may precipitate an outbreak of latent infection.

The seasonal incidence of the disease is quite marked and the majority of the outbreaks occur in chicks from pullet flocks reaching maturity during the late autumn. Morbidity varies from 5 to 50 per cent. of an infected batch.

One can offer little helpful advice at the present time for the control of this disease. There is no drug of value and vaccines reported by American workers have not proved consistently satisfactory. Despite the field evidence for egg transmission, it is difficult in the light of present knowledge to justify a stopping of the sale of hatching eggs from a suspected source and each case must be judged on its merits. In no instance, however, should survivors of an outbreak be retained for breeding purposes or even retained on premises where breeding is carried out. In broiler flocks the only recommendation which can be made is to cull all affected chicks.

The disease produces no visible changes in the organs of affected chicks. Markson and Blaxland (loc. cit.) describe microscopic changes in the cerebellum, spinal cord, pancreas and musculature of the gizzard and proventiculus. They report that it is a routine procedure to eliminate in the first place the possibility of Newcastle disease by the use of the H.I. test, and subsequently to differentiate the condition from other nervous disorders, particularly avian encephalomalacia (crazy chick disease).

(b) Infectious Synovitis

This disease was first reported in Great Britain by Carnaghan (1959) and since then we have encountered a number of outbreaks in broiler plants. The disease was first reported in the United States in 1954 (Olson *et al.*) and since then there has been a rapid spread of this disease and it is now widespread in broiler producing areas in the United States. The outbreak reported by Carnaghan (loc. cit.) occurred in a flock of 5,500 broilers. Losses started at approximately 6 weeks of age, and by 8½ weeks some 320 had died and at least another 10 per cent. were ailing. The majority of the birds examined showed swellings of the joints of the hock and foot and occasionally of the wings; most birds also showed swelling of the sternal bursa and the tendon sheaths between the hock and foot. These swellings contained a grey viscous fluid or creamy yellow material, from which no bacteria could be isolated. When marketed some 30 per cent. of the survivors were downgraded for poor carcase quality.

Experimentally the condition could be reproduced by intravenous, intramuscular, intrasinus, or foot pad inoculation with both the joint exudate and with yolk material from egg passage. Although the disease could not be reproduced by contact, American workers (Thayer *et al.*, 1958) state that egg transmission is indicated.

The confined conditions under which broilers are raised appear to render them particularly susceptible to this disease. In the United States the disease is frequently associated with inter-current infections, particularly the haemorrhagic syndrome and *Escherichia coli* infections.

The agent isolated by Carnaghan appeared to be sensitive to streptomycin and resistant to penicillin, and in chick experiment chlortetracycline in the drinking water at a level of 600 mg./gall. (approximately equivalent to 200 gm./ton) was shown to prevent the development of the disease when given 5 days after infection. American workers (Shelton *et al.*, 1957) also refer to the effective use of chlortetracycline (200 gm./ton). Oxytetracycline was less effective when treatment was delayed until losses occurred, and on withdrawal of treatment synovitis reappeared. Furazolidone has also proved effective in field trials when given at a level of 100/200 gm./ton. (Cosgrove, 1957.)

(c) Infectious Bronchitis

Although this disease was first reported in Great Britain by Asplin in 1948, it has not, in our experience, assumed the serious economic proportions reported in broiler areas in the United States. Most outbreaks encountered appear to have been associated with a depression in egg production and an adverse effect on egg quality in laying birds, and outbreaks in chicks have been comparatively rare. However, Chu (1958a) points out that this may be due to the fact that many outbreaks in chicks and growers can be so mild as to escape notice. The disease, clinically and epidemiologically, is similar to the mild form of Newcastle disease and in all outbreaks showing respiratory symptoms in broiler chicks it is well to eliminate the possibility of the latter.

The disease has a short incubation period of 1 or 2 days and symptoms are usually confined to the lower respiratory tract with coughing, rales and sneezing, sometimes only detectable at night. Where overcrowding and faulty ventilation exist, outbreaks may be complicated by secondary bacterial infections with *E. coli* and p.p.l.o's. In such outbreaks respiratory signs become obvious and appear clinically similar to C.R.D. and coli septicaemia.

There is as yet no evidence of the egg transmission of infectious bronchitis, and recurrence of the disease on the same premises may be due to repeated discharge of the virus from one batch of susceptible chicks to the next.

Post-mortem findings are indefinite and may show little more than small plugs of caseous material in the secondary bronchi with slight clouding and thickening of the air sac membranes.

The diagnosis and differential diagnosis by the isolation of the virus or the detection of antibodies in infected birds are described by Chu (1958b). The disease does not respond to known therapy, and vaccines widely used in the United States are not available in this country. Furthermore, such vaccines have been thought to play a part in the transmission of C.R.D. Control of the disease can be satisfactorily achieved only by breaking the cycle of infection by depopulation and the hatching and rearing of replacement stock in isolation.

(d) Chronic Respiratory Disease

Strictly speaking, this disease should have been dealt with under bacterial diseases, since, in order to clarify the terminology, Chu (1959) suggests that the term should be restricted to the condition associated with pathogenic p.p.l.o's (Nelson's coccobacilliform bodies).

As is the case with infectious bronchitis, this disease, although recorded in the United States as by far the commonest cause of mortality and economic loss in broiler production, does not appear so far to have assumed serious proportions in Great Britain. Nevertheless, a close watch must be kept, since the potential hazards of these respiratory diseases under the conditions prevailing in broiler plants could be extremely serious.

The exact aetiology of C.R.D. is still confused and the position has been reviewed by Chu (1958b and 1959).

Outbreaks in broilers have usually occurred at about 8 to 10 weeks of age. Symptoms consist of marked respiratory distress accompanied by swelling of the facial sinuses, nasal discharge and an interference in growth and food conversion. Coughing, wheezing and marked gasping occur. On post-mortem examination masses of caseous material can be found in the air sacs and in the ocular and nasal sinuses. Although mortality is variable around 10 per cent., it may be as high as 40 per cent. where there is overcrowding and poor hygiene. The main losses, however, occur from the failure of broilers to gain marketing weight, a large number of culls, poor food conversion and the down-grading at processing.

A number of workers claim to demonstrate the efficiency of tetracyclines in the control of C.R.D., and White-Stevens (1957) reported that when chlortetracycline was fed continuously at between 100 and 200 p.p.m. mortality was reduced by half and growth depression prevented. He further reported that the use of this antibiotic at 400 gm./ton for 2 weeks at 6 or 8 weeks of age, followed by 100 gm./ton until marketing was superior to all other treatment and yielded the greatest return over production costs.

Chu (1958a), however, suggests that the drugs have not always been properly used in this country and very often are used at such a low level as not to have much therapeutic value and may render the organism resistant to the drug. He suggests that the drug must be used in effective concentration over a short period. In view of the frequent occurrence of resistant strains in the field, it is advisable to treat a small number of birds as a trial, before flock treatment is carried out.

As in the case of infectious bronchitis, many workers are of the opinion that the most satisfactory control is achieved by the breaking of the cycle of infection by depopulation.

(e) Newcastle Disease

During the first 4 months of 1959 there were 27 outbreaks of fowl pest (Newcastle disease) in broiler plants out of a total of 294 outbreaks confirmed during that period. The majority of these outbreaks occurred in Essex and East Anglia. The number of birds concerned in individual outbreaks varied considerably, from those associated with large broiler units to small groups where small broiler enterprises were being carried out on general farms. Generally, the disease in broiler plants showed a rapid spread and was characterised by marked respiratory and nervous symptoms, with, in some cases, a high mortality rate in the affected groups.

It would seem that broiler plants, because of the frequent contact with processing plants and therefore with personnel and equipment engaged in slaughtering, may be more exposed to potential infection. Obviously, three points are of the greatest importance in the control of Newcastle disease in the broiler industry: (1) the earliest possible reporting of suspected symptoms in order to reduce the transference of infection to central processing plants and thereby to other units; (2) the highest possible standard of disinfection and cleanliness in the equipment used by the processing plants; and (3) reduction to an absolute mimimum of the traffic between broiler units and processing plants.

3. Parasitic Diseases

(a) Coccidiosis

Caecal coccidiosis is still one of the most important diseases in broiler production. Its control, both by husbandry measures and by the use of various drugs, is too well known to warrant repetition. An excellent article reviewing the prophylaxis and therapy of the disease was prepared by Horton-Smith (1956). In our opinion, the most effective drugs for the prevention of the disease are nicarbazin at 0·0125 per cent. (Cuckler *et al.*, 1955) and nitrofurazone at 0·01 per cent. (Horton-Smith and Long, 1952; Harwood and Stunz, 1949). Bifuran, a mixture of nitrofurazone at 0·005 per cent. and furazolidone at 0·0008 per cent. has also given fair though variable results (Horton-Smith, 1959).

For treatment, the sulphonamides—either sulphadimidine in the drinking water (at 0·2 per cent.) or sulphaquinoxaline in the food (at 0·05 per cent.) or water (at 0·0424 per cent)—still appear to give the most satisfactory results when used on the interrupted schedule of treatment basis (i.e., 3-2-3) as described by Davies and Kendall (1954).

Considerable promise was held for the use of synergistic drugs, such as pyrimethamine (Kendall and Joyner, 1956), to potentiate the sulphonamides which would have enabled them to be used at low levels. Unfortunately, however, work would suggest that pyrimethamine is relatively toxic for the chick (Stockdale, 1958; Lucas, 1958).

Glycarbylamide, a drug which has been widely used in the United States, has also shown considerable promise in experimental work at Houghton (Horton-Smith and Long, 1959a), but unfortunately we understand that it is not to be marketed in this country, since there is evidence that resistant strains of coccidia quickly develop. Trithiadole (bithionol and methiotriazamine), Arzene (arsenobenzene) and Zoalene (3,5-dinitro-o-toluamide) are under test, but so far have not shown any marked superiority over the existing drugs.

Edgar (1958) described a so-called vaccine against coccidiosis which involves the infection of the food with small numbers of sporulated oocysts of different

species, followed a fortnight or so later by medication with a coccidiostat. In principle the idea is sound and it attempts to apply what has been learnt from laboratory research to practical control under field conditions. Several workers have criticised the method on the grounds that dangerous numbers of coccidia may be ingested by some still susceptible chickens with their food, that new infections might be introduced on to farms on which "vaccination" is practised, and that similar species of coccidia do not always respond to the same medication.

Intestinal coccidiosis is undoubtedly on the increase in birds maintained on deep litter, and outbreaks are occasionally seen in broilers at or near market age. Although less acute than the caecal type, one species, *E. necatrix*, may give rise to mortality, and both *E. acervulina* and *E. maxima* cause depression in growth and adversely affect food conversion. Recent work (Horton-Smith and Long, 1959b) indicates that these species, responsible for outbreaks of intestinal coccidiosis, respond differently to the various drugs in common use and this fact may account for the conflicting results sometimes observed. In these workers' experience, *E. necatrix*, for example, responds more effectively to nicarbazin and nitrofurazone, whilst these drugs are inferior to the sulphonamides in dealing with *E. acervulina* or *E. maxima*. It would appear, therefore, that in order to obtain the most effective results in the treatment of the disease, it may be necessary to identify the species involved rather than to refer to the disease under the composite title of "intestinal coccidiosis".

Prophylactic control of intestinal coccidiosis is unlikely with present drugs to be highly effective, since the compounds commonly used for the caecal type are not particularly effective against *E. acervulina*.

It should be unnecessary to comment on the association between outbreaks of coccidiosis and litter management. Davies and Joyner (1955) state that dryness is the only lethal factor of importance in reducing the number of parasites in deep litter. They also comment on the seasonal variation in litter moisture and the associated coccidial population, the highest number of oocysts being present during November and February when temperatures obtained in deep litter are not sufficient to eliminate oocysts and ova and may even favour their development.

Further field observations by Payne (1959) showed that studies of broiler houses in Southern England have shown that, when litter is unduly wet, excessive ventilation in winter is usually associated with a high relative humidity in the house, which leads to deterioration of the litter and this, in turn, may lead to a high mortality.

For the disinfection of broiler house floors and walls after the removal of infected litter a 10 per cent. solution of household ammonia used as a wash or spray is still the most effective compound known. Care should be taken, however, that the operators are properly aware of the possible toxicity of ammonia and they should be provided with respirators. Where litter is to be re-used for subsequent crops, it has been found that the stacking of the litter into heaps approximately 6 ft. high and 10 ft. in diameter effectively produces temperatures varying up to 160°F. which destroys oocysts and worm ova. The heap should be turned after 4 days and left for a further period of one week before being respread (Horton-Smith and Long, 1954; Long and Binstead, 1959). Fresh litter, however, should always be used under and around the brooders for the first few days. The depth of litter is of some importance, since it has a diluting effect on the parasite population and should be not less than 4 in.

(b) Blackhead (Histomoniasis)

Essentially a disease of the turkey poult, this is now appearing with increasing incidence in broiler chicks, although, generally speaking, the actual losses do not reach the proportions normally seen in turkeys. Few outbreaks in our experience have as yet reached dimensions which would warrant either prophylaxis or therapy; nevertheless, there is published evidence to show that both furazolidone and acinitrothiazole are equally effective in the chick as in the turkey (Horton-Smith and Long, 1956a, b). One might hazard the opinion that the increased incidence of this disease in broilers may possibly be associated with the increased *Heterakis* burden under certain deep litter conditions.

(c) Roundworms

A recent article (Reid, 1958) suggests that it is highly debatable whether or not it is economically worth while to worm broilers, since worms are seldom likely to be present in sufficient numbers to affect weight gain or mortality, although there is some evidence in America that a heavy worm burden may adversely affect mortality from other diseases, particularly infectious bronchitis. Reid points out that it is the larval stage of the worm which causes symptoms during its migration into the intestinal wall, and assuming that a chick picks up infection on the first day of life, mature worms which produce eggs will not be found until the thirtieth day. If the husbandry has been good, the number of larvae during this period will be so small as to be negligible, and it is therefore the second migration cycle from the mature worms' eggs which can cause trouble. This cannot start until the female is old enough to lay eggs and the eggs have matured. The minimum time for an entire life cycle is 35 days and it is impossible, therefore, for more than one such cycle to be completed during the period of a 10-week broiler and the birds are marketed before the second crop of worm eggs are produced by the second generation of females.

Thus worming, if it is considered necessary, must be aimed at the removal of the first generation of females before they reach maturity. There are, therefore, limited periods when dosage would be effective. Worming before the twenty-ninth day is a waste of money, as is the use of drugs within 12 days before marketing. Treatment about the fifth week probably achieves the best results.

Piperazine compounds marketed in this country as adipate, citrate and bisulphide, at a level of 100–500 mg./kilo body weight were highly effective in the removal of *Ascaridia galli* (Horton-Smith and Long, 1956c).

(d) Ectoparasites

Severe infestations with external parasites have been comparatively rare in our experience in broiler houses. There are today, however, a variety of compounds available for the control of such parasites, i.e., gamma benzene hexachloride (Lindane), piperonylbutoxide/pyrethrins (Pybuthrin), or 0, 0-dimethyl dithiophosphate of diethyl mercaptosuccinate (Malathion). These are marketed in forms to be used either for the treatment of individual birds—not likely in broiler production—or as aerosols or for mixing in the litter, i.e., 1 per cent. Lindane applied as a single application to the litter at the rate of 3 lb. per 1,000 sq. ft. (Blount, 1955); piperonyl butoxide/pyrethrin can be successfully employed in a low-pressure aerosol dispenser or from high-pressure aerosols (Brander and Thomas, 1954); or Malathion at a level of ½ lb. per 20 sq. ft. sprinkled in the latter gives good results (Harding and Quigley, 1956).

One point with regard to mites is of some interest, at least to us. We have recently received a number of requests for advice on the control of "mites" in broiler houses, the history given being that the litter is heavily infested and that the "mites" are observed both on the birds and on attendants. On examination these "mites" have been identified as free-living forage mites of various species, such as *Caloglyphus rodionovi* and *Haemolaelaps casalis*, and they are not known to be pathogenic. It is debatable whether it would be economic or necessary to make any serious attempt to control them.

Environmental Diseases

(a) Keratoconjunctivitis

Barber (1947) first observed that 25 per cent. of a group of chickens kept under poor hygienic conditions developed an ulcerative keratitis and severe conjunctivitis when about 16 weeks old. The concentration of ammonia in the rooms in which these birds were kept was so strong as to cause the paint to peel from the walls. The removal of the diseased birds to clean, well-ventilated rooms resulted in their rapid recovery. Following on these observations, a number of reports of a similar condition occurring in broiler plants in the United States have been made. In this country, Carnaghan (1958) referred to 5 outbreaks in broiler chicks reared on previously used litter. Symptoms were first noticed in chicks between 2 and 3 weeks of age. The estimated incidence varied from 2 to 30 per cent., although the mortality rate was generally low. The main symptoms were that the birds stood with closed eyes and appeared dejected. The feathers on the back and between the wings were damp and matted due to the birds rubbing their eyes in an attempt to relieve the irritation. In one house of 10,000 broilers it was estimated that 3,000 were affected. The eyelids were swollen and congested and there was some haemorrhage observed on the corneal surfaces of the eyelids, and ulceration could be seen on the cornea.

Transmission experiments appeared to rule out the possibility of an infective agent and the exposure of chicks to ammonia fumes caused lesions similar to those seen in the field.

(b) Cannibalism

Blount (1959) states that cannibalism, a natural result of overcrowding, may occur at any time after the first fortnight, unless steps have been taken to debeak chicks or darken the windows.

(c) "Chilling" (Blount loc. cit.)

When the general room temperature is too low, "chilling" may occur sporadically during the first 6 weeks, actual deaths probably being due to smothering. On post-mortem examination the specimens are squashed, frequently decomposed and characterised by congestion of the lungs and nephritis.

(d) Haemorrhagic Disease

Next to coccidiosis, this is probably the major cause of mortality in broiler plants. Unfortunately, the exact cause of this disease is still obscure, although there is an extensive volume of literature on the subject.

On first appearance the disease may be confused with acute caecal coccidiosis. The affected birds huddle together, shiver, may pass blood in the droppings and die quickly. In many cases death occurs without symptoms. In a number of birds, haemorrhages may be observed under the wings, in the legs, comb and feet, but frequently the haemorrhages are internal or on the muscles and only observed on post-mortem examination. Mortality rises rapidly after the first few cases and it may be as high at 50 per cent., gradually subsiding after 10 days. The condition appears to clear up within 2 or 3 weeks. On post-mortem examination there are multiple haemorrhages in the subcutaneous tissues, particularly on the breast, thighs, on the wall of the intestinal tract and in the heart and spleen. A constant lesion is the degeneration of the red bone marrow of the long bones, indicating acute anaemia.

Cover *et al.* (1955) reports that so far efforts to reproduce the disease experimentally have given inconsistent and somewhat inconclusive results. These workers suggest that the disease is not associated with a deficiency of vitamin K.

Forgacs and Koch (1955) claim to have reproduced the condition in chicks fed on grain which had been inoculated with eight selected fungi isolated from feed from boiler houses.

Barnett *et al.* (1957) suggest that a haemorrhagic condition could be reproduced by the addition of 0·05 to 0·09 per cent B.A.P.N. (beta-aminopropionitrile) in the diet, the haemorrhages being increased by the addition of 0·15 or 0·3 per cent. sulphaquinoxaline. Haemorrhages increased when soya meal was replaced with protein meal and also when 10 per cent. fat was added to the ration.

Although coccidiostats, particularly the sulphonamides, are usually associated with outbreaks, they would not appear from experimental work to be primarily responsible for the toxicity, and even massive doses of these drugs do not produce the clinical condition as seen in the field. The exact factors, therefore, which, in addition to the use of coccidiostats, produce the clinical disease, are still obscure.

The only advice that one can offer at the moment, when an outbreak occurs, is to withdraw the coccidio-

stat and to avoid, particularly during hot weather, an excessive intake of medicated water. In some outbreaks it has appeared that intercurrent diseases, e.g., blackhead, and stress factors such as caponisation, may precipitate the disease. One cannot emphasise too strongly the need for accurate diagnosis before rushing into drug treatment. In many cases of haemorrhagic disease the history has stated that treatment was started because of an outbreak of coccidiosis, and yet on examination no evidence of coccidiosis was found in the flock.

The fact that haemorrhagic disease is fairly common does not invalidate the use of coccidiostats, since the number of outbreaks must be small compared with the widespread use of these drugs. It is difficult at the moment to suggest why outbreaks should follow the use of the drugs in certain instances and not in the majority of cases and this may be associated with certain environmental or even genetic factors.

Nutritional Diseases

On the whole, deficiency conditions are relatively uncommon in broiler plants, although individual cases of perosis and dermatitis occur from time to time. The former is generally associated with a deficiency of manganese and the latter with a deficiency of pantothenic acid or biotin. Compounded rations are seldom deficient in these nutrients, and it may be that the few cases seen are either in chicks with a higher requirement for these substances or those which have suffered from a reduced consumption of food by overcrowding or lack of trough space. *Nutritional roup* (Avitaminosis A) is also relatively uncommon in broiler chicks submitted for examination.

Crazy Chick Disease (Nutritional Encephalomalacia)

Although this disease is considered to have a nutritional background, it probably falls within a slightly different category, since the exact aetiology is still obscure. Markson *et al.* (1957), in discussing the aetiology of the disease, suggest that a diet truly deficient in vitamin E will seldom in itself cause encephalomalacia, but that the disease can be caused by the oral administration of highly unsaturated fats and that an adequate intake of the tocopherols (vitamin E) can protect against these effects. Antioxidants, e.g., DPPD (diphenyl-p-phenylenediamine), also protect against destruction of vitamin E. Proprietary chick foods compounded by a number of manufacturers were implicated in the cases examined by these workers, but the majority of samples of proprietary foods examined contained a higher level of tocopherol than was present in the mash fed to the control chicks. There was little evidence that outbreaks were influenced by the deficiency of tocopherol or the presence of cod liver oil in the ration.

The condition usually develops in chicks between the ages of 3-6 weeks and is characterised by drowsiness and uncoordination. Chicks stagger and fall on their sides. They exhibit a pedalling action of the legs, may walk in circles and show a contraction of the head over the back or between the legs. Symptoms are much more marked than those which occur in epidemic tremor.

On autopsy the cerebellum may appear swollen, soft and smooth in appearance, with visible capillary haemorrhages. The histological findings are described by Jungherr (1949). Recent work referred to by Duckworth (1958) indicates a possible relationship between vitamin E and selenium in the aetiology of the condition.

In a paper of this type one cannot hope to cover all the diseases encountered in broiler chicks, and an attempt has been made to discuss those which, in our experience, appear to be of major importance. The low mortalities so far recorded in Great Britain must not give rise to a false sense of security, since it must be borne in mind that the broiler industry in this country is of very recent development and the majority of the housing and equipment has been in use for a comparatively short time. A close watch must be kept on the disease picture, since there is every indication from the course of events in other countries that the rapid passage of crops through broiler plants, particularly if associated with an increase in population density, must play an important role in the build-up of infection and subsequent increase in the incidence of avian diseases, particularly those of a respiratory nature.

The high standard of husbandry and housing which has obviously been achieved in the larger broiler units in this country must be maintained, since there is evidence from our own experience that the more serious outbreaks occur in units where management is at a low level or where an attempt has been made to break into the broiler market by the conversion of existing, and usually unsuitable, buildings.

There are still obvious gaps in our knowledge concerning the aetiology and epidemiology of avian diseases, particularly as they occur under this type of husbandry.

There is an equal if not even more pressing need for information on the environmental requirements of the broiler chick.

Although not necessarily relevant to this paper there is obviously a need for a close scrutiny of sanitary conditions associated with the processing and marketing of poultry products.

References

ASPLIN, F. D. (1948). *Vet. Rec.*, **60**, 485.
Avian Diseases (1958). **2** (4), 543.
BARBER, C. W. (1947). *Cornell Vet.*, **37**, 349.
BARNES, E. (1957). *Roy. Soc. Hlth J.*, **77**, 446.
BARNETT, B. D., RICHEY, D. J., and MORGAN, C. L. (1957). *Poult. Sci.*, **36**, 1104.
BARR, F. S., and CARMAN, P. E. (1957). *Southeastern Vet.*, **IX**, 11.
BLAXLAND, J. D. (1954). *Proc. Xth W.P.S.C. Edin.*, p. 210.
——, SOJKA, W. J., and SMITHER, A. M. (1958). *Vet. Rec.* **70**, 18, 374.
BLOUNT, W. P. (1955). *Vet. Rec.*, **67**, 1087.
——. (1959). *Mod. Poult. Kpg.*, **80**, 19, 14.
BRANDER, G., and THOMAS, P. (1954). *Proc. Xth W.P.S.C.*, p. 286.
CARNAGHAN, R. B. A. (1958). *Vet. Rec.*, **70**, 35.
——. (1959). *Vet. Rec.*, **71**, 81.
——, and SOJKA, W. J. (1958). *Vet. Rec.*, **70**, 645.
CHU, H. P. (1958a). *Vet. Rec.*, **70**, 55.
——. (1958b). *Vet. Rec.*, **70**, 1064.
——. (1959). *Proc. 16th I.V.C. Madrid.*

COLES, R. (1959). Personal communication.
COSGROVE, A. S. (1957). *J. Amer. vet. med. Ass.*, **130**, 287.
COVER, M. S., MELLEN, W. J., and GILL, E. (1955). *Cornell Vet.* **45**, 366.
CUCKLER, A. C., MALANGA, C. M., BASSO, A. J., and O'NEILL, R. C. (1955). *Science*, **122**, 244.
DAVIES, S. F., and JOYNER, L. P. (1955). *Vet. Rec.*, **67**, 193.
DAVIES, S. N., and KENDALL, S. K. (1954). *J. comp. Path.*, **64**, 87.
DUCKWORTH, J. (1958). *Conf. Sci. Prins. of Feedg. Farm Livestock*, p. 1. (Rearing and Table Poultry.)
EDGAR, S. A. (1958). *Proc. XIth W.P.S.C., Mexico.*
FORGACH, S. J., and KOCH, H. (1955). *Poult. Sci.*, **34**, 1194.
GOLDEN, E. F. (1953). W.P.S.A., December, 1953.
GORDON, R. F. (1951). *Proc IXth W.P.C., Paris*, Sect. 3, 95.
——. (1956). *Ag. Review*, **1**, (10) 23.
——, and TUCKER, J. F. (1957). *Brit. Vet. Jour.*, **113**, 99.
——, GARSIDE, J. S., and TUCKER, J. F. (1959). *Vet. Rec.*, **71**, 300.
HARDING, W. C., and QUIGLEY, G. D. (1956). *J. Econ. Entomology*, 49, 806.
HARWOOD, P. D., and STUNZ, D. I. (1949). *J. Parasit.*, **35**, 175.
HARRY, E. G. (1957). *Vet. Rec.*, **69**, 1433.
HORTON-SMITH, C. (1956). *Vet. Rec.*, **69**, 8 (11), 164.
——. (1959). Personal communication.
——, and LONG, P. L. (1952). *Brit. Vet. Jour.*, **108**, 47.
—— and ——. (1954). *Proc. Xth W.P.S.C., Edin.*
—— and ——. (1956a). *J. comp. Path.*, **66**, 1, 22.
—— and ——. (1956b). *J. comp. Path.*, **66**, 4, 378.
—— and ——. (1956c). *Poult. Sci.*, **35**, 3, 606.
—— and ——. (1959a). *J. comp. Path.*, **69**, 2, 192.
—— and ——. (1959b). *Brit. Vet. Jour.* In press.
JUNGHERR, E. L. (1949). *Ann. N.Y. Acad. Sci.*, **52**, 104.
KENDALL, S. B., and JOYNER, L. B. (1956). *Vet. Rec.*, **68**, 119.
LEVINE, P. P. (1959). Personal communication.
LONG, P. L., and BINSTEAD, J. A. (1959). In press.
LUCAS, J. M. S. (1958). *Nature*, **182**, 449.
MARKSON, L. M., and BLAXLAND, J. D. (1955). *Vet. Rec.*, **67**, 131.
—— and ——. (1958). *Vet. Rec.*, **70**, 1208.
MARKSON, L. M., CARNAGHAN, R. B. A., and PARR, W. H. (1957). *Brit. Vet. Jour.*, **113**, 303.
Min. Hlth & Pub. Hlth Lab. Serv. Mon. Bull. (1959). **18**, 26.
OLSON, N. O., BLETNER, J. K., SHELDON, D. C., MUNRO, D. A., and ANDERSON, G. C. (1954). *Abst. Ann. Meet. Poult. Sci. A.*, 39.
PAYNE, C. G. (1959). *Agriculture*, **65**, 629.
REID, W. M. (1958). *Broiler Growing*, Sept.
——. (1959). Personal communication.
SHELTON, D. C., BLETNER, J. K., OLSON, N. O., ANDERSON, G. C., and WEAKLEY, C. E. (1957). *Poult. Sci.*, **36**, 114.
SHRIMPTON, D., BARNES, E., and MILLER, W. (1958). *J. Sci. Food Agri.*, **9**, 353.
SMITH, H. W. (1955). *Vet. Rec.*, **67**, 749.
SMITH, R. C. (1959). Personal communication.
SOJKA, W. J., and CARNAGHAN, R. B. A. (1959). Personal communication.
STOCKDALE, H. G. (1958). *Vet. Rec.*, **70**, 720.
TEMPERTON, H. (1953). *Harper Ad. Col. Conf.*, Sept.
THAYER, S. C., STROUT, R. G., and DUNLOP, W. R. (1958). *Poult. Sci.*, 37, 449.
WALKER, J. H. (1957). *Lancet*, **II**, 283.
WHITE STEVENS, R. (1957). *Vet. Rec.*, **69**, 217.
WILLS, F. K. (1955). *Southwest Vet.*, **8**, 2.
WILSON, J. E. (1955). *Vet. Rec.*, **67**, 894.

THE SPEAKER'S INTRODUCTION

The Chairman, Mr. J. Edwardson, introduced the author and invited him to present the paper.

Dr. R. F. Gordon, in presenting his paper, said that he appreciated the opportunity to do so. It was always a pleasure to speak to a veterinary audience on poultry problems. The broiler industry was fascinating. Probably no branch of agriculture had expanded at the speed of the broiler industry's expansion, nor had any development in livestock husbandry reached such a degree of intensivism. These two factors had led many of them to fear catastrophic outbreaks of disease. Although accurate mortality records were difficult to obtain, the evidence so far would suggest that in this country the mortality level on the whole had not been as excessive as they had prophesied, although obviously serious outbreaks were encountered from time to time on individual plants.

It was pointed out in the paper, however, that the broiler industry was of comparatively recent origin and since many of the plants and buildings and much of the equipment were new one must still issue a warning that the continuous population of such plants, accompanied by the tendency towards increasingly large unit size, might materially alter the picture.

Furthermore, the profit margin in broiler production was such that, quite apart from killing diseases, any sub-clinical condition interfering with optimal growth, food conversion and carcase quality could have serious repercussions on the industry as a whole and could well turn a producer's profit into a loss.

Dr. Gordon then showed a number of slides.

The first one showed the mortality experienced on various official poultry tests carried out in the U.S.A., from which it would be seen that mortality ranged at different centres from approximately 4 per cent. to 11 per cent. The range of mortality in stock entered by individual owners varied from as low as 1 per cent. to as high as 30 per cent. Two British surveys showed that in one case, based on 1¼ million birds, mortality averaged 4 per cent. (range 2 per cent. to 9 per cent.) and in the other, carried out on 33 flocks, the range varied from 1 per cent. to 14 per cent., the average being approximately 5 per cent. It would appear, therefore, that so far the average mortality in Great Britain was somewhat lower than that in the States, but one must again repeat the warning that the industry was a comparatively new one.

The second slide showed that in the U.K. broiler industry, at present estimated to have an output of 100 million birds annually, and an average mortality of 4 per cent., the monetary loss would be in the region of £¼ to £1¼ million depending on the age at which the birds died. Figures obtained from research workers in the United States indicated that in that country the approximate loss was 72¼ million broilers out of a total of approximately 1,500 million annually. In the State of Delaware alone this mortality meant a financial loss of approximately 7 million dollars or £2½ million. Estimated figures from the U.S.A. put the cost of disease per 1,000 broilers, including cost of vaccination and medication, at between £16 and £18. A similar effort to estimate the economic loss from mortality in the cost of production of 1,000 broilers in this country suggested a figure which would vary between £5 and £15 depending on the age at which mortality occurred.

Estimates which had been made by the British Broiler Growers' Association based on 100 birds indicated that, assuming a profit margin of around 6d. to 9d. per bird, with an 8 per cent. mortality in the last week or so, the producer was only just covering his costs of production; while if the figure rose to, say, 12 per cent. a loss would be incurred.

The effect of sub-clinical disease on cost of production was well illustrated by the fact that in two recent investigations no mortality was experienced by the producers, with the result that no action had been taken until the crops in question were marketed, when the percentage of second-grade carcases rose from the usual level of 10 per cent. to 30 per cent. and it was estimated by the group that this had resulted in a financial loss on the birds concerned. As a matter of interest, the investigation indicated that the loss of carcase quality was associated with a low-grade coccidial infection. Occult outbreaks of that type could be far more costly than an acute killing infection in the early stages, since the producer had already incurred the cost of feeding and rearing the crop concerned. The price received for a down-graded bird was more than 1s. less than that received for a grade A bird. Therefore, there was a loss of at least 3d. per pound plus some loss on body weight. If the total cost of producing a bird was 70 pence, a 10 per cent. mortality or downgrading during the last weeks would eliminate 7d. profit.

It was difficult to indicate the incidence of the diseases most commonly encountered in broiler plants or to estimate their relative economic importance.

An attempt had been made, based on *post-mortem* records, to list the diseases in the order of their occurrence, compared with a similar table of losses recorded in Delaware, the most densely broiler-populated area in the States. The illustrative table, however, must be treated with discretion since in one case it was difficult to separate chicks received from broiler producers from those received from non-broiler flocks, whilst the Delaware figures included birds up to as old as 5 months of age. Nevertheless, it would be seen that there was a distinct similarity, the commonest conditions being respiratory diseases, Salmonella infections, coccidiosis, epidemic tremors, fowl paralysis, infectious synovitis and the haemorrhagic syndrome.

The next slide was probably somewhat more realistic in that it had been compiled from Dr. Gordon's records for the last 3 months of the current year. The pattern had altered and it would appear from experience that at the present the most important diseases were coccidiosis, both caecal and intestinal, Salmonella infections, epidemic tremors, respiratory diseases, haemorrhagic syndrome, crazy chick disease, fowl paralysis and infectious synovitis. One must also bear in mind that those figures were of limited value since the histories received did not indicate the loss incurred in any particular outbreak. It might well be that figures from other laboratories differed since there was a tendency for laboratories to draw from certain geographical areas and therefore from specific broiler groups.

Following compilation of the table, the figures for July 1959 in chicks between 0 and 10 weeks showed a further 53 outbreaks of coccidiosis, 23 outbreaks of salmonellosis, 17 of haemorrhagic disease, 11 of encephalomyelitis, 14 respiratory infections, 15 yolk sac infections, 13 other bacterial infections including *coli* septicaemia and 10 infectious synovitis. The increase in the latter disease was of some importance and would be dealt with later.

Another slide had been produced by Dr. Blount and showed in chart form the periods during the 10 weeks of broiler production at which those various conditions tended to exert their effect, compared with the normal mortality levels experienced where no epidemic disease was present. Dr. Blount suggested that initially a mortality of about 2 to 2½ per cent. was normal during the first 10 days of life, the mortality figure thereafter varying between ½ to ¼ per cent. for the remaining period.

In the paper a description was given of some of the more common diseases met with in broiler plants, together with the present methods for their control, but in the time at one's disposal for introducing the paper one could do no more than refer briefly to some of the salient points concerning these conditions.

One was rather perturbed at the increasing incidence (at least it was so in Dr. Gordon's own experience) of salmonellosis in view of the potential public health hazard involved. Although the majority of outbreaks recorded in his own case had been caused by *Salmonella thompson* or *S. typhimurium*, a number of exotic species had been encountered in recent years. Whether there was any connection between that and the recent reports from the Public Health Laboratory Service that Salmonella organisms were present in imported animal foodstuffs it was difficult to say. Work in progress at Houghton showed that the continuous use of tetracyclines in feeding stuffs gave rise to the emergence of strains of *S. typhimurium* of increased resistance to the antibiotic used. The exact significance of those observations had yet to be established.

In the paper reference was made to a form of arthritis associated with a variant strain of *S. pullorum*. Carnaghan (1959) had informed Dr. Gordon that some 15 further outbreaks of that disease had been encountered and in a large number of those a standard strain of *pullorum* had been isolated from the lesions, whilst in 2 outbreaks *S. typhimurium* appeared to be the casual agent. He, Carnaghan, also reported two outbreaks of fowl typhoid in broiler chicks in which the mortality level was high.

The question of bacterial resistance was probably of greater veterinary importance in regard to avian coliform infections and recently a number of outbreaks of *coli* septicaemia had been observed in broiler chicks. The isolates in most instances were found to be resistant to tetracyclines. Although the mortality rate was seldom higher than 10 per cent. the disease resulted in an increase in the number of carcases down-graded at marketing. Symptomatically the disease showed some resemblance to C.R.D. and it was the accumulation of caseous material in the abdominal and thoracic air sacs which resulted in the rejection of the carcase. There was some field evidence that the disease might be associated with over-crowding and faulty ventilation. Field trials suggested that treatment with furazolidone had given promising results. Carnaghan (1959) reported that in recent outbreaks of *coli* septicaemia diagnosed at Weybridge a few of the strains of *E. coli* isolated showed some sensitivity to tetracyclines *in vitro*. It was also of interest to note in relation to that disease that in 2 outbreaks Newcastle disease was also confirmed.

A disease which had recently made its appearance in Great Britain and which was likely to become of importance if it followed the pattern seen in the U.S.A. was infectious synovitis. Again, mortality might be fairly low, around the 10 per cent. mark, but, like *coli* septicaemia, it occurred in birds of 6 to 8 weeks of age and resulted in anything up to 30 per cent. of the survivors being down-graded. The disease was characterised by lameness and swelling of the sternal bursa. American workers stated that egg transmission of the disease might occur, whilst outbreaks were frequently associated with inter-current infections, particularly *coli* septicaemia or haemorrhagic disease. Work in Great Britain indicated that chlortetracycline given in the drinking water 5 days after infection effectively prevented the development of the disease.

An important point had ben reported by Carnaghan (1959) which should be remembered in assessing the importance of infectious synovitis, namely that in 1958 only 8 outbreaks of the disease were encountered at Weybridge, whereas for 1959 the total to date had already reached 43. It would be noted from the slides prepared from Houghton records that whilst no outbreaks were recorded in 1957, in the second 3 months of 1959, 7 outbreaks were seen and in July 1959 there were 10 outbreaks, giving a total of 17 outbreaks for the 4 months April to July 1959. The disease was obviously becoming of major importance. Recent literature on it indicated that the best results in the treatment of the condition were achieved with chlortetracycline.

So far the respiratory diseases, particularly chronic respiratory disease and infectious bronchitis, so commonly referred to as being the main cause of mortality in the broiler plants in the U.S.A., did not appear to have assumed such serious proportions in this country. Those diseases, together with their control, were referred to in the published paper and it would be remembered that they were the subject of a detailed review given by Dr. Chu (1958).

It was of interest to note that Newcastle disease (fowl pest) had also made its appearance in broiler plants and during the first 4 months of the current year there were 27 outbreaks in a total of nearly 300 confirmed during that period. The majority of those occurred in Essex and East Anglia. Although the number of outbreaks might appear to be relatively small, the actual number of birds involved was high since certain large units were involved. The disease tended to spread rapidly under broiler conditions and in many instances the mortality rate was fairly high being characterised by marked respiratory and nervous symptoms. The potential risk of fowl pest infections in broiler plants needed to be stressed in view of the close and frequent contact with processing plants, their equipment and the personnel engaged in slaughtering.

Despite the notable advances which have been made in the prophylaxis and therapy of coccidiosis, the disease could still be regarded as amongst the most important causes of economic loss. The various drugs now available were discussed in the paper and one would merely repeat that in the opinion of the author and his co-workers the most effective drugs for the prevention of the disease were nicarbazin and nitrofurazone, whilst for treatment the sulphonamides gave the most satisfactory results when used on the "intermittent schedule" basis of 3-2-3.

During the last year or so new drugs such as glycarbylamide, trithiadol, arzene, zoalene and unistat had made their appearance on the American market. Some were under test

in Great Britain, but so far had not been found to be markedly superior to existing drugs. One looked forward with considerable interest to an extension of the work on the use of synergistic drugs to potentiate the sulphonamides. A good deal of interest had been aroused by the use of vaccines for coccidiosis, but workers in Great Britain were still critical of that method of control.

There was little doubt that intestinal coccidiosis was increasing in birds kept on deep litter and outbreaks towards the end of the broiler period could have serious economic repercussions. Methods for the prophylactic control of intestinal coccidiosis had yet to be evolved and it was pointed out in the paper that recent work indicated that for the successful treatment of the condition identification of the species involved should be attempted since drugs varied in their efficiency against different species.

It was of importance to note that fowl paralysis was occurring at an increasingly early age and a number of outbreaks, some of them accompanied by a high mortality, had been observed in broiler plants in chicks as young as 4 to 6 weeks of age. It would seem that broiler conditions favoured the transmission of the disease. Reports from America indicated that certain forms of leucosis were occurring with increasing incidence in broiler plants and were giving rise to considerable concern.

A condition which appeared to some extent to have accompanied broiler production was that known as the haemorrhagic syndrome, for although outbreaks occurred in other classes of stock it was commonest in broiler plants, possibly as a result of the invariable use of coccidiostats in broiler feeds. Levine (1959) stated that in the Eastern States of N. America the disease, once of major importance, had now practically disappeared and he in turn related that to the replacement of sulphaquinoxaline in poultry feeds by the newer coccidiostats. The disease, however, was an interesting one whose aetiology still remained obscure.

On the whole, deficiency diseases were comparatively rare as a cause of large-scale loss in broiler plants, mainly due to the care which had been given to broiler nutrition by the compounding industry. Nevertheless, in large units individual cases occurred from time to time exhibiting typical symptoms or lesions of such conditions as vitamin A deficiency, rickets, perosis, etc. The exception to that comment was probably so-called crazy chick disease and although it was thought to have a nutritional background and had been stated to be associated with a vitamin E deficiency the exact aetiology was uncertain. Suffice to say that in some instances the use of antioxidants appeared to control the condition.

Environmental diseases undoubtedly played a big part in broiler production. Conditions such as kerato-conjunctivitis, which was alleged to be associated with excess ammonia fumes, smothering, chilling, clogged beaks, lamenesses of uncertain actiology, were all encountered from time to time. On the whole, such conditions could not be satisfactorily diagnosed by the laboratory worker since lesions were uncertain and histories invariably inadequate.

In those conditions, where a definite diagnosis could not be arrived at by the usual laboratory techniques the problem could often be resolved by a visit to the plant concerned and an investigation of the environmental conditions and management. There was little doubt that closer collaboration between the veterinarian in the field and the laboratory worker could do much to reduce both mortality and subclinical disease in broiler plants.

Although a few of the larger broiler groups might well be in the position to employ their own veterinary advisers it would seem that in that expanding industry, in which there was undoubtedly a large amount of capital invested, the veterinary profession as a whole must play an ever-increasing part if that section of the industry was to continue on an economic basis.

In addition to the control of diseases, that new development of what had been termed "agri-business" brought with it a host of new problems bordering on the veterinary field, calling for studies in relation to environment, housing and ventilation, processing, marketing and meat inspection, and the use of food additives with the possible long-term side-effects on the epidiomology of avian diseases.

References

Blount, W. P. (1959) *Mod. Poultry Kpg. 80,* 19, 14.
Carnaghan, R. B. A. (1959) *Personal communication.*
Chu, H. P. (1958) *Vet Rec.* 70, 1064.
Levine (P. P. (1959) *Personal communication.*

The Opener

Dr. N. S. Barron, in opening the discussion, said it was only to be expected that a new facet of agriculture and of their work as veterinarians should be included in the deliberations of the Congress. The Organising Committee were to be congratulated not only on that but on persuading Dr. Gordon to present the paper. Though already well established, the broiler industry was new to this country but, alas! like all projects involving the growth and production of livestock and livestock products, disease exacted its toll. Thanks to Dr. Gordon and a small group of enthusiastic workers the profession had not been found lacking in giving the industry strong support.

As they could read in the paper under discussion, with the formidable hazards to which the broiler was exposed they could not be complacent. Although a number of the diseases to which broilers were heir were well understood, their behaviour under exceptional conditions—one might almost say spectacular surroundings when one considered the concentration of birds, ventilation, dim lighting and high feeding levels—were not always predictable. Also, there were new diseases creeping in such as epidemic tremors and synovitis. There were always new problems associated with a new form of intensive stock keeping. The establishment of a concentrated community on litter was indeed intriguing.

The expression "built-up litter" was now in common use. It seemed to him that there was an analogy here with the soil. As a result of bacterial action both were transformed texturally and nutritionally if conditions were right, the one for poultry, the other for plants. A higher rate of growth and generally better viability were to be expected in chicks on old, built-up litter irrespective of diet. They presumed that was mainly due to the increase in microflora.

In litter of vegetable origin, as distinct from sand or pebbles which they might see more of if shavings got any scarcer, moulds and yeasts tended to increase up to 8 weeks from the day they were first used and coliform and lacto bacilli established themselves. The pH changed from 6.3 to 8.0 as litter aged. Liming at the rate of 10 to 15 lbs. per 100 sq. ft. markedly reduced living organisms and brought the pH back to 6.8. Could they afford to ignore those factors? How important were they?

Would Dr. Gordon comment on the need for changing litter and the frequency? There were Coccidia and perhaps an occasional worm ova to reckon with. Was there anything else? What about the moulds and yeasts? Did that background picture bring the haemorrhagic syndrome, already well known in this country, more into focus?

The table by Dr. Blount included in the paper and illustrating the wave of diseases to which broilers were prone might well fill one with alarm and anxiety if it were not for the work on poultry diseases already done which made it possible for many of them to be tackled now. One need only refer to the drug treatment of salmonellosis and coccidiosis of caecal and intestinal origin. Fowl paralysis was shown in the table as capable of giving a brief kick as the broiler reached maturity at 9 to 10 weeks.

What was really disturbing was the importance of nephritis on this scale with its resultant cannibalism. What suggestions as to aetiology had Dr. Gordon here? Heaven forbid that in the present day and age those changes should be nutritional—that wall which collected so much mud! Were the moulds interfering? Could the bacterial content of the egg as such have an influence on kidney function?

The profession had always insisted that the control of disease and the preservation of health came squarely within its prerogative. That had not excluded poultry, but because of the peculiar hole and corner way in which the poultry industry had, in the main, conducted its business in the past the matter had not been a paying proposition for those in the profession, other than the research worker. They were now seeing large units—very large units—which might well

afford the services of a veterinarian, but what was lacking was not a knowledge of the diseases but of the problems of poultry husbandry. Dr. Gordon had quoted the cost of losses. The saving in preventing those losses might pay the veterinary surgeon's bill, especially if the cost of the all-essential laboratory diagnosis was kept within its present limits.

The General Discussion

Mr. A. C. L. Brown (Chelmsford): "It was not my intention to speak at this meeting but one of the slides shown by Dr. Barron prompts me to rise.

"I refer to the slide on which a very eminent broiler producer is seen among his birds demonstrating litter texture; this gentleman it was noted was wearing his ordinary walking shoes and every-day suit.

"Newcastle disease in broiler units raises new problems for both the veterinary profession and the broiler industry. Normally in ordinary poultry flocks fowl pest is suspected and dealt with by the Animal Health Division at a stage when a comparatively few birds are affected. This is not possible in broiler units. Fowl pest in broiler units appears first with a slight increase in the daily death rate which rapidly mounts from about 10-20 birds to 50 or perhaps 500 birds by the third day. By the time infection can be reported and dealt with many thousands of birds are infected and the virus produced pours out of the ventilators and air extractor fans in much greater amounts than has previously been experienced in the epidemiology of this disease in this country.

"The broiler producer, therefore, has a dual responsibility —firstly, to protect his own flock and to limit the constant procession of people who call to advise him and move among his birds for one reason or another and, secondly, to realise that if he is careless to any degree and becomes involved in an outbreak of fowl pest he is putting all the other flocks in his district to a very great risk of infection.

"One final point is that the best control measure that can be recommended to the broiler industry is the keeping, on any broiler unit or farm producing broilers, of birds of one age group only. The value of this has already been demonstrated in Essex where one large broiler group became involved in fowl pest. Infection was certainly limited within this group by the fact that the majority of the producers in the group kept one age of bird. Dr. Chu in his remarks on infectious bronchitis amply demonstrated the value of this system.

"One of the major contributions the veterinary profession can make to the broiler industry is to stress to producers that security must come high on the list of practices which are introduced to increase the efficiency of the industry. Security can do much to reduce not only fowl pest but also many of the other diseases so competently dealt with by Dr. Gordon in his paper and Dr. Barron in his opening of the discussion."

Mr. N. Comben (Southall): "I believe that this is the first time that the disease problems confronting the vast and relatively new broiler industry have been discussed by such a veterinary gathering. This discussion is not before its time. Broilers are big business today, and considerable fortunes are tied up in breeding and growing units. With broiler houses holding up to 32,000 birds apiece it is easy to understand how many hundreds of pounds may be lost overnight when disease, or the result of some management fault, strikes such a flock. A timely word of advice can frequently obviate such losses, and the man on the spot who can offer such advice will find that his services are much in demand.

"At the present time the broiler industry is serviced largely by the free advisory services offered by various ancillary trades. I am sure, however, that the majority of us who are concerned with such services will welcome the day when this work is more commonly accepted as a routine part of general country veterinary practice. I would commend this discussion to all present, for the broiler industry offers a very interesting scope to our profession in work which can be both satisfying and rewarding.

"I should like to crystallise one point which has been indicated both by Dr. Gordon and by Dr. Barron. Broilers grow very rapidly, and as fast working organisms they must be regarded as being delicate. When everything about their breeding, feeding and environment is as it should be they will give their expected high performance. But it takes very little to upset this balance, and it is well to remember that a density of infection or infestation which might not necessarily affect slower growing birds, may have a far more marked effect on a broiler.

"If one follows the evolution of the broiler industry in this country one sees how closely this follows behind the changes which have occurred in the parallel industry in the U.S.A. This is unfortunately also true of broiler diseases. I believe that all the indications suggest that the next major disease problem we shall have to tackle in the broiler industry will be the respiratory diseases which are already the cause of serious losses in the U.S.A., where vaccination programmes are now being adopted. Infectious bronchitis and C.R.D. in particular are now becoming far more common in laying and breeding flocks in this country, and I should like to ask the speakers if they have any views about measures which could be adopted now to lessen the risks of the spread of these diseases during the next few years."

Dr. D. Luke (Belfast): "With reference to epidemic tremor, I take it that diagnosis was based on the presence of the characteristic lesions in the brain and viscera. Has Dr. Gordon any comment to offer on those conditions in chicks which resemble epidemic tremor clinically but in which no macroscopic lesions can be detected histologically?

"Salmonellosis presents many facets and a survey of feeding stuffs recently completed in Northern Ireland showed the presence of about 12 exotic serotypes in samples of compounded feed. Some of these types, notably *S. orion* and *S. infantis*, were recovered from the faeces of healthy poultry, yet during the recent chick rearing season we did not isolate any of these species from chicks examined *post-mortem*.

"The appearance of the leucosis complex in broiler flocks, especially where there is no exposure to adult birds, is interesting. Has Dr. Gordon any comment to make on this point?"

The Reply

Dr. Gordon, in replying to the discussion, said he was gratified by such stimulating contributions as had been made, though most of the answers would have to be, "I don't know." Dr. Barron had referred to the management of deep litter. In the early stages in America some 10 years before, it was suggested that the control particularly of parasitic infections was concerned with the amount of ammonia produced in litter working well; but it had been shown that the factor was the degree of dryness rather than ammonia, and not only dryness but texture of litter at the surface. Some of the worst outbreaks of coccidiosis occurred where caking had been allowed to take place. He had been in broiler houses where the litter was like concrete and the freshly passed droppings remained on the surface.

There was a lot to be done not only concerning types of litter but in terms of turning it at regular intervals. Change of litter was bound up with scarcity of litter. In some areas that had become extremely serious. Wood shavings appeared to give the best results and they were now in very short supply in some broiler areas. Some work must be done to find alternative methods. In one house wire floors were used rather than deep litter. Depth was important. A depth of 6 in. or 8 in. was recommended.

Dr. Gordon said he had recently visited a large broiler plant in Europe and shallow litter of about ¼ in. to 1 in. was being used on asphalt floors. There was said to be no coccidiosis, presumably owing to the dryness of the floors and the greater ease in keeping the shallow litter moving. An alternative method was to put the litter in conical heaps, leave it for about 4 days and turn the outside to the centre, re-spreading after 3 or 4 days.

As to dryness affecting subsequent bacterial action, in some instances a light spraying of the outside of the heap had been recommended.

Reference was made to non-specific conditions. One of

the slides had referred to about 500 examinations but there were another 300 cases where it could not be established that the cause of mortality was disease. Where the type of outbreak was possibly related to husbandry, to the kind of house, ventilation rates and so on, the laboratory worker was rather helpless and there was room for co-operation between the veterinary practitioner or field worker and the laboratory worker.

Dr. Gordon said he did not know the answer to the question on nephritis. Poultry research was relatively new. Broiler production was even newer and the biggest gap in their research was physiology, though it was hoped that in future physiology of the kidney would be included in the programme.

Some of the causes of cannibalism were bad lighting in the house, high humid atmosphere, poor feathering. A number of breeders were paying particular attention to feathering in order to overcome cannibalism.

He agreed with all the points raised by Dr. Luke. He saw cases with symptoms resembling epidemic tremor or crazy chick disease but in which no histological lesions could be found on examination. One or two outbreaks of Newcastle disease had come to light.

With regard to the exotic Salmonellas, where the exotic strains were recovered there did not appear to be any tendency for them to become established in the breeding flocks or broiler units. It could be that an outbreak was due to the few chicks which had access to contaminated food, and there it seemed to stop.

The American workers maintained that true fowl paralysis was probably not egg-born. It was denied that there was any evidence of egg transmission, but in view of the epidemiology it was difficult not to feel egg transmission must be occurring.

As to the fumigation of houses with aerosols, there was evidence of their efficiency in the case of empty houses. There did not appear to be much advantage in using aerosols in houses where birds were in occupation, except possibly in the case of ectoparasitism. If one used an aerosol machine with formaldehyde it was a fairly effective and quick method of dealing with the atmosphere of the house. Some broiler producers used formaldehyde with the addition of formalin to potassium permanganate. There had been two quite big fires in broiler houses where the formalin had been put into huge drums, usually oil drums, and then the potassium permanganate was thrown into the formalin. The results were devastating. Appropriate warning was given by the fire authorities.

As to unhygienic processing plants, some of the bigger groups were putting up ideal plants. They were usually extremely efficiently run and hygienic. Then, one had seen small plants where the small producer attempted to do his own processing, and even some of the egg packing stations, where standards of hygiene left much to be desired. Birds ran about the floor of the house and blood and faeces and so on were trampled into the floor and there was very little effort in personal hygiene on the part of the workers. At one fairly big packing station Dr. Gordon had seen outside the collecting door a pen of pigs being fed on the viscera of fowls—just to keep the cycle going.

Apparently there was no grading laid down in Great Britain though there was in the United States. Usually the first-grade fowl was broad breasted and had white flesh. A second-grade bird had a triangular shaped breast and very often had bruising, blood splashing and so on.

The figures he had given related to individual parcels received It was suggested that caecal coccidiosis incidence was higher than had been indicated. That was probably true. It was difficult to arrive at definite figures, but it was fairly certain that the better producers were quite conversant with symptoms, the appearance of blood in the faeces, and made their own diagnosis in the case of coccidiosis.

Vaccine in the long-term might be the right answer. Work was in progress on immunity against coccidiosis, but some of his colleagues were not very enamoured with the vaccines produced by Edgar in the United States.

Reference had been made to the vast number of people who seemed to populate broiler plants. Some of the big producers, such as Geoffrey Sykes, who felt it was a mission in life, were so proud that they spent their lives taking people round their houses. But did the "continuous traffic" referred to by the questioner relate to veterinary officers, foodstuff representatives or drug manufacturers.

What was said about the one crop system seemed positively right. In the earlier stage the marketing side was not geared up for the one-crop system, but the producers had grouped themselves together into what was called vertical integration.

Dr. Chu asked whether the industry were aware of the need to control disease and that research work required money. Dr. Gordon could only say that if they were not then he would have been sacked by Dr. Wooldridge 11 years before!

The Chairman, in proposing a vote of thanks, said they were extremely indebted to Dr. Gordon for a big contribution to their practical knowledge of broilers. They were grateful also to Dr. Barron.

Plenary Session

The Relationship Between Intensive Agriculture and Animal Disease

BY

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I NEED not try to say how very greatly I appreciate your invitation to present an address on "The Relationship Between Intensive Agriculture and Animal Disease". That you should have asked me to do this, rather than someone else, I have taken to signify a wish not for anything highly scientific, but for something in the nature of a summary of the position as it is at the present time. That is what I have endeavoured to prepare.

The key to the understanding of this relationship lies in the interdependence of all forms of life. Animal life depends upon vegetable life, and it is only green plants in the presence of sunlight that are able to fashion their vital substances from the elements of the earth.

To think of such things has been the worry, or the pleasure, of a very small minority in the past, where our forbears endeavoured, by such agricultural procedures as they knew, to make two blades grow where one grew before. It was a procedure that in the beginning depended on the sweat of the brow of the husbandman in the midst of his rural surroundings, but that has gradually become complicated until in our days it employs the cerebrations of the atomic physicist. Meanwhile, the pleasant pastoral association with shepherds' pipes and the song of the cuckoo seems to have been left far behind. With all this, however, the lesson of maturity is of how little we know—and in the agricultural field this refers outstandingly to the complexity of inter-relationships between living things.

There may be some difficulty for those who are not concerned with ecology in appreciating the permanence of the set order of natural things and I propose, therefore, to give two pictorial illustrations; at one end of the scale of time the picture of an early association of parasite and host that has persisted throughout evolutionary periods into our own era, and at the other end a picture of our own unwanted guests, the hangers-on that were with us in forests and in cave dwellings and are still with us in our hygienic cities and modern homes.

The Antiquity of the Partnership of Host and Parasite

My first picture is of the whale as a land animal, walking through the mist of the early morning, feeding on the succulent herbs in company with the early representatives of our sheep and cattle. He coughs repeatedly, a familiar, husky cough. He is a young animal and is suffering from a mild attack of parasitic bronchitis due to the presence of lungworms.

This is not pure romance; it is a picture constructed on sound evidence that the lungworms now carried by that leviathan who forges through the seas have descended from the same companions that harassed his primitive ancestors when they consorted with land animals so very long ago. Here is the evidence: whales, porpoises, seals and sealions have their lungworms at the present time, closely related to those occurring in our domestic sheep and cattle: this kind of parasitic lungworm is found only in mammals and not in any other kind of creature: zoologists agree that mammals originated on the land: can there be any doubt, therefore, that these sea mammals became acquainted with their lungworms on the land and took them into the sea when, in the slow course of evolutionary time, they began to live there?

The impression I wish to convey by this first parasitological illustration is of the immense time through which these hosts and parasites have lived together in mutual tolerance, and according to the rule that the whale must no more upset the lungworms than the lungworms must upset the whale.

The Persistence of the Partnership of Host and Parasite

The second picture concerns the atomic age, and a group of atomic scientists and others are meeting in the Cockcroft Hall at Harwell to discuss the use of gamma rays for the sterilisation of foods. One of their number presents a paper on the application of this new method to the destruction of parasitic worms transmissible through meat to man. He points out the interesting anachronism that some of these humble companions of ours, who have travelled along with us through the immense stretches of evolutionary time, who belonged to our wild habitations in forests and caves, and hut dwellings, who are despised and miserable creatures, should still be with us, and should accompany us to the meeting in the Cockcroft Hall. He agrees that most of the unwanted hangers-on of this kind have now been suppressed and are no longer admitted to our social affairs, but points out that two or three species of parasitic worm are still regular attenders at meetings in our highest places and without doubt are represented at that meeting of atomic scientists.

Now that we are become so sanitarian and, in our own conceit, so advanced beyond our fellow creatures, we like to forget these early associates whose presence reminds us of our humble beginnings. It is regarded as indecent, therefore, to mention the tapeworms and roundworms which still accompany civilised man about his daily affairs. Surely there could be no better

example of the firm establishment of the ecological order of things than this association of the atomic scientist and the parasitic worm.

The Microbial World

The parasites just mentioned are things that may be seen by the naked eye, and by their very size and form appeal to our perceptions, but there is a fundamental substratum that can only be seen through a microscope. Because it is out of sight and can only be appreciated by thought—which is a troublesome process—the microbial world is out of mind, and we forget that we have to maintain our mammalian lives amid such a hoard of competitors, an organisation of organisms, which occupies to the full every possible living space on earth. In their studies of decomposition the soil bacteriologists find a continuous succession of microbial populations. As the particular organic substrate most suited to an existing population is changed by the microbes in occupation, it is made more suitable for another class which then takes possession because its enzymal activity is better fitted, so that it ousts its predecessors. In so doing, however, the substrate is changed and, in its turn, the dominant organism is replaced by another, and yet another, in a long sequence of microbial populations each appropriate to the ecological niche prepared by its predecessor.

It is into such a world that the germ-free offspring of our domestic animals and birds are borne to begin their separate existence and fill the place that is their birthright. It is not a mere matter of adaptation, however, to a hostile environment; rather is it an integration with the whole biological world, often beneficial and sometimes indispensable to the larger organism. The leguminous plants and their nitrifying bacteria is a good example from the vegetable kingdom, and from the animal kingdom the ruminants and their microbial co-operators over digestion. There are, in fact, many special designs of stomach, seen to the greatest perfection in the ruminant, but all concerned with prolonged retention of undigestible food in order that microbial populations living there for their own ends may carry out a predigestion that is of the greatest value to the host.

It has been surprising to find that during the first two or three weeks of life, foals (and doubtless other young animals as well) eat the fæces of the dam and thereby acquire the inoculum for the development of the proper micro-flora in their intestines. In this context coprophagia must be regarded as highly hygienic, as it is essential to the health of an animal whose well-being is integrated with that of a complex microbial community.

To follow these chains of dependence certainly leads to unexpected conclusions. Their investigation also calls for some strange procedures; see the lengths to which Reyniers and his co-workers had to go in order to separate experimental animals from their microbial associates! An annotation in *The Lancet* describes the procedure which begins with the aseptic removal of the fœtus from the maternal uterus. The article then goes on to say:

> "The larger rearing units are very elaborate structures consisting of several rooms arranged in two storeys. The three men working in these chambers wear completely sealed diving-suits of plastic material, provided with 40 ft. of stainless-steel air-lines. On entering the chambers the operators are hermetically sealed in their diving-suits and are then given a shower-bath in antiseptic for fifteen minutes. They are then totally immersed for half an hour in tanks of 3 per cent. formalin containing detergent. Any imperfections in the diving-suit soon become apparent, either by the escape of bubbles or by the effect of the formalin on the occupant of the suit. The operators then climb a ladder from the antiseptic tank through a trap-door, where they are met by a shower-bath of sterile water. They are then ready to attend to the sterile animal colony."

Normal processes are arrested in this "purified" world, cleared of all bacterial organisms; dead animals fail to putrify and excrement is odourless. Animals reared there from germ-free parents are not, however, prepared to face the natural world. On one occasion, through some fault in the isolation technique, the benign and friendly organism *Lactobacillus acidophilus*, used in commerce for the souring of milk, found its way into the pure culture of isolated hosts and caused an epidemic of disease that proved fatal to many of the unprotected animals.

This needed protection is acquired by the normal animal in response to the antigenic substances of the bacterial world, and the adaptive mechanism, not only of the host species but of each individual, is moulded as the individual grows, to fit into its particular microbial environment. Can we wonder that such complications are easily disarranged!

It is only when we cease to accept things as ordinary and use our imaginations to find new points of view that we can add to our understanding, and in endeavouring to see the effects of intensive agriculture we must try to look at the situation from every side. There is a *hostism* to be studied as well as a *parasitism*, and a *defence of the parasite against the host* as well as of the *host against the parasite*; and, if we widen the meaning of the word just a little, there is *a disease of an environment* as well as a *disease of the individual animal* within it.

The Changing Face of Agriculture

It is only since the Second World War that research has been recognised as an occupation of ordinary individuals. The scope for the application of the work of these people to agriculture is limitless, so that numbers of the new discoveries of the zoologist, botanist, geneticist, physicist, chemist, engineer and veterinary worker have an immediate bearing and provide something new for the progressive agriculturalist to try. Many of these trials imply an alteration in the balanced community of living things, often recognisable to us as disease.

The successive steps in the process of intensification are made alternately by the small group of research workers, handling theoretical considerations in laboratories, and a larger group of veterinary clinicians, in close touch with the practical farmers. These observers report evidence showing that the even tensions among living things have been deranged in a new direction. The research group then try to find the reason and an adjustment is made, often in the form of an additional artificiality. An important point to

observe is that these adjustments are in no way permanent; they must be constantly repeated or the tensions again become uneven and disease appears. It is, in fact, an artificial world of plants and animals that must be maintained and not a natural one that maintains itself.

I will endeavour to deal with some of the things that we have learned by these trial-and-error methods under three headings, "Artificial Animals", "Artificial Foods" and "Artificial Microbial Background". Before proceeding, however, it might be advantageous to refer to the tendency for a change to take place in the position of the veterinary clinician, who is concerned less and less with individual animals and to an increasing degree with groups. He deals with subclinical disease among flocks and herds, looking for the first signs of departure from normal health and into the fine points of adjustment that would maintain the balance. His clinical knowledge is employed in detecting these early changes and determining their cause, and for this he deals with individuals; but his treatment, both prophylactic and therapeutic, most commonly concerns the whole group.

Artificial Animals

The slow process of selective breeding has already modified the anatomy and physiology of our domestic animals and birds with the object of enhancing production. Some of the evil consequences of these deviations from the natural are familiar, such as the susceptibility of the mammary gland of the cow to bacterial invasion, or of the fleece-covered sheep to the fatal disease of sheep scab. These changes had happened prior to the introduction of intensive agriculture and are familiar, but the more recent developments have brought with them an entirely new set of troubles, concerned, strangely enough, with the successful suppression of bacterial pathogens. The usual way for an animal to develop its defence mechanisms against the microbial world is to receive antibodies from the maternal parent before birth takes place, or soon afterwards, through the colostrum. The newly born animal, thus passively protected against the first contacts with pathogens, is given a breathing space in which actively to provide its own more powerful antibodies.

It is this essential step in the development of a normally resistant adult animal that we oppose by the continuous use of antibiotics in early life, depriving our artificial animals of their normal preparation for life in a world of microbes. The danger was perhaps exaggerated when first it was recognised, but we must be just as careful now not to underestimate the possibilities that accompany the introduction of each new and powerful protective agent. The use of cocciostats in overcrowded poultry houses is a good example, it being necessary to allow for some controlled development of the parasite, or the chickens die of coccidiosis as soon as the artificial protection is withdrawn. The new parasiticides and protective vaccines against parasitic bronchitis are still in their trial period, but would provide another example if their efficacy were to approach completion. Wherever the reintroduction of a pathogenic organism cannot be avoided, we must keep in mind the danger of producing susceptible flocks and herds through any means that may be relinquished by the farmer when the disease is no longer troublesome. The use of such preventive means must continue so long as the parasite persists, otherwise our artificial animals fail.

Artificial Foods

In the earlier period of our scientific agriculture the agromonists were satisfied with the investigation of naturally-occurring herbage plants to discover which species produced the most digestible herbage. The next step was to improve each useful species by judicious selection and controlled pollenation. Through this means, numerous strains were produced, far exceeding the normal grasses in nutritive value, so that we were provided not only with artificial pastures for the artificial animals to graze, but artificial plants growing there as well. In theory, this is all to the good. Even extensive field trials, however, may fail to reveal what ultimately becomes only too plain to the agriculturalist and his veterinary adviser that all is not well. An excellent example comes from the antipodes, were, in parts of marked climatic variation, the successful development of animal husbandry has depended upon four pasture plants, all of which are now associated with specific disease problems—an outcome of the monoculture. Wild white clover is associated with bloat, perennial rye grass with facial eczema and rye grass staggers, *Phalaris tuberosa* with Phalaris staggers, and subterranean clover with prolapse of the uterus and infertility. All are prevented by the substitution of a mixed herbage, such as more nearly approaches the natural.

There are also practical considerations that operate against the optimum utilisation of the wonderfully nutritious grasses that have been developed in our own country for pastures—the animals prefer not to eat them! At least, it is so in many instances, where they are seen to ignore the luscious green sward that looks so appetising in our eyes and prefer to graze the benty growth of inferior grasses and weeds that line the hedgerows. It is thought to be a matter of palatability; and here I should like to ask the research people the reason for our not using the appropriate sense organ in the investigation of this point. I can imagine the son of some expert wine taster, after serving his apprenticeship, entering a veterinary laboratory in order to apply the same methods to the recognition of the flavours of grasses, and after some years of practice on filtrations from the pulverised leaves (with occasional reference to the cow) being able to recognise bovine appreciations and know what is of good vintage. He would then direct the course of research towards improvement of strains that would not only please the analyst but the cow as well.

Hypomagnesæmia may be mentioned as a clear-cut example of the association of disease with artificial pastures. In recent years its connection with rapidly growing herbage has been clearly recognised, and widespread outbreaks are sometimes seen to occur with seasonal variations in the weather, such as bring about a rapid growth of herbage. The prevalent occurence of the disease in the south-western counties dur-

ing the late summer of 1958 was an example of this. An association also exists with the intensive grassland systems of ley farming and the increased use of nitrogenous fertilisers such as force a rapid growth of lush herbage. How such grass causes hypomagnesæmia is not clear, and the suggestion of an association with excessive amounts of ammonia in the rumen and a consequent depression in the absorption of magnesium can be regarded as little more than a working theory.

These ills, associated with artificial foods, are examples of situations in which clinicians report on the type of disturbance following the introduction of intensive methods and on the circumstances under which they occur. In several instances we know of some practical way of avoiding the loss. The precise reason for its occurrence and for the operation of the remedy often, however, remain a problem for the laboratory worker. It was through investigations into such a point that we were led to the knowledge that a trace of cobalt is an essential part of the diet of ruminants, in order that their microbial associates may synthesise a complex substance, vitamin B_{12}, that is essential to the life of the host animal, but can only be obtained through the activities of certain bacteria in their intestines, working in the presence of cobalt. This vitamin does not occur in plants; it is necessary for it to be synthesised by bacterial action—which reflects again the essential inter-relation between the complex vertebrates and the bacterial environment in which they live, and shows the breadth of the field that confronts the veterinary investigator.

Artificial Microbial Background

Continuing with our consideration of the disturbances caused by our intensive agriculture, in the natural biological world we come to a very interesting chapter, introduced by the isolation of penicillin. Some indication of the importance of the normal microbial associates of domestic animals had already been obtained in connection with digestion in stomach and intestines, but it was not until this new kind of substance empowered us to interfere in a big way with bacterial populations that we began to see the true nature of the *status quo*.

This field for investigation is an exciting one. It was the farmers who hurried along to make practical use of the new discovery and mixed antibiotics with the animals' food, and it was highly stimulating for the bacteriologists to observe a more marked effect on some farms than on others. It was not the pigs that differed, nor the places in which they were kept, nor the food or management, but the microbial background of the environment from which each successive litter acquired its intestinal flora. Animals that we had considered to be perfectly normal and healthy were revealed by this means to have been growing at a suboptimal rate because of slight differences in the microbial populations of their intestines and the environment in which they were living.

It is interesting that the first evidence should have been of favourable possibilities through interference by the use of these newly discovered substances. We have since seen more of the possibilities for evil. There is a long list of reported intestinal ills and pathological conditions due to organisms that cause no trouble at all, so long as the normal tensions are maintained. These are new upsets that belong only to our artificial world. There are a number of organisms that may be regarded as on the fringe of pathogenicity, in the position of waiting to occupy living space, but kept in abeyance by competitors and host resistance. The powerful new antibiotic substances, in suppressing some components of the bacterial populations of the intestines, alter the ecological balance and a pathogenic role is taken on by organisms that in the ordinary way are quite harmless.

There is still plenty of romantic exploration to be carried out in research, but anyone who seeks real adventure in a research career should go into virology at the present time. The exciting discoveries here are tumbling in too rapidly for anyone who is outside the specialised field to keep abreast. Whoever browses through the reports of new observations, however, gets an impression of a background of viral entities in which tensions exist. There are normal and abnormal associations between vertebrates and their viral environment, as well as the microbial environment that has been discussed.

The romantic appeal in this work is reflected in the use of the term "orphan viruses", described as "viruses in search of a disease". Numerous examples of these entities have been recognised since the development of tissue culture methods and a new cult has sprung up among the virologists who fill papers increasingly with references through initial letters to M.H.V. (mouse hepatitis virus), E.E.E. (eastern equine encephalitis) and the host of enteric cytopathogenic orphan viruses, E.C.H.O., E.C.M.O., E.C.B.O. and E.C.S.O., enteric cytopathogenic viruses of human, monkey, bovine and swine origin respectively. Each of these groups contains a dozen or more recognisable strains, some only of which are so far known to be capable of producing pathological effects in living animals. More than sixty different viruses of this kind have already been isolated from the intestinal tract of the cow alone. The veterinary ecologist may well ask what possibilities are there here for upsetting the stable viral background and with what effect?

Eradication of Pathogens

The road ahead, of increasing intensification through trial-and-error procedures, entails continuous readjustment for the deranged ecological relationships. It looks like an uneasy course. There is, however, one bright spot on the horizon of the future, concerning those essential pathogens that it may be possible to eradicate from our island country. Here is another field of activity for the veterinarian of the future.

This idea of eradication is in the air at the present time, through our outstanding success in the tuberculosis campaign, and a word or two might be said about the general prospects, concerning eradication of the helminth parasites of grazing animals. There is a special association between helminthic disease and intensive agriculture, because the population of the

parasites is linked with the concentration of animals on pasture ground. As the intensity of the use of improved grassland increases, therefore, we must expect increasing trouble from parasitic worms.

The present occasion is not a suitable one to enter into detailed considerations, and I must be content with the conclusions reached—that the eradication of some of the worst kinds of parasitic worms from almost any farm is within the bounds of possibility, but only at very great trouble and expense. In view of the virtual certainty of reintroduction, it would therefore be unwise to make the endeavour, and quite impracticable at the present time to eradicate the parasites from the country as a whole. For each group of these parasites a time will come, however, when the question of relative costs will have to be carefully worked out; should we continue this recurrent expenditure on control measures, along with the inefficient use of our improved pastures, or should we make one concerted and expensive onslaught and endeavour to eradicate the parasite?

The time for this may come along more quickly than we expect, as in the outstanding example of the eradication of anophiline mosquitoes from large islands and from large areas of mainland, through the use of DDT; or the complete elimination of the nodular worm of sheep from the eastern provinces of Canada by the use of phenothiazine during the period of their winter housing. It is not unlikely that it will be the discovery of new anthelmintics of unheard-of efficiency that will ultimately enable us to proceed with the eradication of parasitic worms.

Until that time arrives, however, we must learn how to live with them, by the use of such parasiticides as we have and by the adaptation of husbandry methods. By this I do not mean to advocate complacency, introduced by the repetition of such slogans as "happy though infected" or "learn to love your liver flukes", but that it would be a mere waste of nervous energy to attempt eradication until there is reasonable prospect of success. It is only when that time comes that we should release our adrenalin and do the best we can.

The control methods now available are not without hope, and it is a great encouragement to see the complete disappearance of some of our serious problems through husbandry changes alone. Syngamiasis is a case in point. At one time this was perhaps the most serious disease with which the rearer of chickens had to contend. It completely disappeared, however, with the introduction of the intensive system of rearing, and a generation of poultry keepers has arrived which has only heard of the condition as part of the history of the past. Trichomoniasis in cattle is another example of the same kind. In this instance it was the introduction of artificial insemination that was responsible. The same sort of thing might happen with the development of "zero grazing", or, if we look still further into the future, in the "three-dimensional" agriculture that is to come, when grazing animals no longer see the green fields but are kept indoors, in buildings of ever-increasing height, and their food is brought to them free from fæcal contamination.

Underlying Principles

The appearance of new forms of disease associated with artificialities in the environment is an essential element of progress in animal husbandry. One cannot remove pieces from a jig-saw puzzle, or pull the threads of an embroidery, without spoiling the pattern —yet that is what we do in suppressing whatever seems to be unwanted, and developing only what we desire in our intensive agriculture. The limitless variety of living things filling every available space are necessary to one another in the fabrication of a complete and permanent biological world.

Most of these forms of disease represent only a temporary hindrance to progress, as once the deranged detail has been ascertained we are able to make the necessary adjustment. It is, however, recurrent and we must expect new diseases to accompany successive steps in progress.

Our difficulty in understanding and our inability to proceed by anything other than trial-and-error methods arise through the complexity of the numerous forces in operation. Evidence of this difficulty is seen in the use of such vague and indefinite terms as "balance", "tension" and "background", where we should like to be specific. There is also the human difficulty of looking at things objectively. It is essential to take the objective view if we are to make the best use of our limited powers of understanding—and here again we find evidence in the use of terms. The word "parasitism" is used in a familiar fashion by the specialists who, however, make no use of the word "hostism", and yet those two are complementary ideas and represent two aspects of one and the same phenomenon. "Hostism" is the parasites' point of view and is of equal importance with "parasitism" in understanding the relationship between the two.

The phenomenon of parasitism must not be regarded as curious and exceptional, nor parasites as occasional and odd; even in the diversity of their form, they are more numerous than the hosts on which they live. Not to take their point of view into account is unreasonable. There are good hosts with which a parasite community may live happily and there are bad hosts that react in an unfavourable way and produce disease in the parasite community—it is more than a parallel case; it is an identical one.

This situation may be sensed most readily by the worker with the gross parasites, where the onset of sickness can be seen in each individual; female nematodes "go off their lay" and young cestodes cease to grow as they ought to do. The larger parasitic worms assume an individuality, on which account it seems natural to study their well-being and to see the mirror-image of the host's disease. In this situation, "resistance" and "adaptation", "competition" and "co-operation" are seen to be facets of one and the same phenomenon. There are sound reasons for thinking that, given the necessary technical skill and the ability to provide for the needs of the parasite, it would be possible to manufacture vaccines to protect their young against the harmful effects of the host, just as it is possible to do the reverse. Such a duality of operational forces is less easily conceived among a rabble of bacteria or in a background mist of viruses—and yet

the situation is the same and similar responses must be in operation.

It is through gropings such as these that we find the principles which serve to guide us towards an understanding of natural things, where the complexity of detail merely confuses.

In a story entitled *Letting in the Jungle*, Kipling described the rapidity with which a forest village disappeared when once man had left it. The same applies to our intensive agriculture and to all artificial animals, artificial pastures and artificial microbial background; they cannot be maintained except by constant attention, and in the end we find, paradoxically, that the jungle is the only orderly arrangement of living things.

Reference

"Special Article", 1953, Germ-free Life. *The Lancet*, October 31, 933-4.

The Therapy of Parasitic Gastro-Enteritis in Sheep and Cattle

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SUMMARY—The value of phenothiazine and a number of newer anthelmintics has been assessed for the treatment of parasitic gastro-enteritis. Phenothiazine, in spite of many disadvantages, is probably still the best all-round anthelmintic, although further work may demonstrate the superiority of certain of the newer compounds. The strategic and tactical use of anthelmintics is considered and the value of continuous low-level phenothiazine treatment is discussed.

PARASITIC gastro-enteritis is caused by some six or eight species of trichostrongylid nemotode, some of which are found in the abomasum and others in the small intestine. *Haemonchus, Trichostrongylus* and *Ostertagia* are the genera represented in the abomasum, whilst *Trichostrongylus, Bunostomum, Cooperia* and *Nematodirus* are those commonly found in the small intestine. The species vary a little as between cattle and sheep, but these specific differences are of no consequence so far as treatment is concerned. *Chabertia ovina* and *Oesophagostomum* sp. which occur in the large intestine should also be included in the parasitic gastro-enteritis complex. The pathogenesis of the several species of worms involved has not been completely studied in pure infestations, but sufficient work has been done to indicate that the disease caused by different worms is a distinct entity and parasitic gastro-enteritis is really a complex of diseases—haemonchosis, ostertagiasis, trichostrongylosis, etc. Sometimes in a field outbreak one species of worm predominates, and a special case of this is the disease in young lambs called nematodiriasis. At other times a mixture of several species is involved.

The therapy of parasitic gastro-enteritis is, therefore, the treatment of some half a dozen diseases, one or more of which may be present at any one time. Clearly, the ideal drug for this purpose is one which will completely eliminate the several species of nematode involved and which is also completely non-toxic for both cattle and sheep. No drug yet discovered has all these attributes and much energy is at present being expended by pharmaceutical research workers in the hope of discovering something nearer to the ideal. Failing a drug of wide activity, a series of drugs of specific activity would serve, but such a solution to the problem would necessitate exact diagnosis, by clinical means only, or with the aid of laboratory procedures. Indeed, exact diagnosis is essential if the best use is to be made of those drugs at present available. Complete elimination of worms is, however, not desirable where drugs are used for prophylaxis, and the ideal drug for this purpose would remove the majority of the worms, but leave behind those stages responsible for the production of immunity.

Although copper sulphate, copper sulphate and nicotine sulphate mixture, tetrachlorethylene and copper-arsenic mixtures are still used in some parts of the world for the treatment of parasitic gastro-enteritis, they were largely superseded some two decades ago by phenothiazine. Phenothiazine has the advantage of a much wider range of activity than the anthelmintics it replaced, although it cannot be regarded as ideal, for it is practically ineffective against *Bunostomum phlebotomum, Cooperia* spp. and *Nematodirus* spp. Its activity against *Haemonchus contortus* and *Trichostrongylus* spp. makes it the most useful anthelmintic we have at present for use in parasitic gastro-enteritis of cattle and sheep. It suffers from the disadvantage, however, of low efficiency against immature worms, necessitating repetition of treatment, even in the absence of reinfection, to ensure the elimination of worms which develop to maturity subsequent to the initial treatment.

Of recent years it has become evident that the particle size of phenothiazine preparations exerts a profound influence on anthelmintic activity. As early as 1940 Gordon had shown a coarse preparation of phenothiazine to be less efficient against *Oesophagostomum columbianum* in sheep than a finely ground commercial preparation. Gibson (1951) was able to detect no difference in activity between six commercial samples of the drug of varying particle size, but work carried out since then has shown that even his coarsest sample was fine enough to be highly efficient. In more detailed studies Gordon (1956) demonstrated that for full efficiency the particles of phenothiazine should not exceed 30μ and for high efficiency the majority should be below 20μ. Other workers, Whitten (1956), Thomas and Elliott (1957) and Kingsbury (1958), have confirmed the influence of particle size on the action of phenothiazine, and from their work it seems clear that full efficiency against *Trichostrongylus* spp. is attained only when samples of the drug having the majority of the particles below 10μ are used. However, extreme fineness does not in all cases give maximum efficiency, for Gordon (1958c) reports that a fine preparation having 50 per cent. of particles below 2μ gave the lowest efficiency so far recorded with phenothiazine against oxyurids in mice. Thomas and Elliott (1957) suggest that the ideal phenothiazine preparation has 70 per cent. of particles under 5μ or 90 per cent. under 10μ with the remainder of the particles up to 30μ. Full investigation of this subject is not yet complete and it may be found that there is an optimum particle size for different worms or perhaps for worms inhabiting different parts of the alimentary tract. At present the indications are that samples having fine particles are more efficient against para-

sites in the abomasum and small intestine (*Haemonchus* and *Trichostrongylus* spp.), whilst coarser samples are more efficient against large intestinal parasites. It seems that a proportion of both should be present in the ideal sample of phenothiazine.

In sheep, phenothiazine seems to be almost without toxicity. In early trials Taylor and Sanderson (1940) gave as much as 400 grams to a lamb, without ill effect, and field experience since that time has demonstrated the safety of the drug. The only untoward effects reported in sheep, apart from trauma in dosing, have been a few cases of abortion (Hungerford and Howe, 1944; Warwick, Turk and Berry, 1946), but experience has shown that administration of the drug to pregnant ewes is safe except during the last three weeks of pregnancy. In cattle, however, toxic effects have frequently been reported. Taylor and Sanderson (1940) and Roberts (1941) both record fatalities in calves, and since then many other records have accumulated. The occurrence of toxicity in cattle is quite unpredictable. Many thousands of animals have been treated, sometimes with high doses, without ill effect, yet on other occasions animals succumb after a small dose. Weak and debilitated animals are the worst risk and it is prudent to treat selected animals from a herd to test the susceptibility to phenothiazine before embarking on treatment of the whole group. Another form of toxicity in cattle has been recorded by Whitten, Clare and Filmer (1946), who describe a photosensitisation following treatment with the ordinary therapeutic doses. Swelling occurred round the eyes accompanied by profuse lachrymation and corneal opacity, with temporary blindness which lasted about ten days. Subsequent work—Whitten and Filmer (1947)—showed that the trauma occurs between twelve and thirty-six hours after dosing and that the substance responsible is a metabolism product of phenothiazine—phenothiazine sulphoxide. This trouble can be avoided by protection from sunlight during the day following treatment.

Two minor defects of phenothiazine are its rather bulky dose and the fact that its oxidation products cause undesirable staining of the wool. This latter defect, however, can be largely overcome by the use of a dosing gun incorporating the McMaster oesophageal tube which ensures that dosing can be carried out without spilling any of the material. For sheep, phenothiazine is probably still the best anthelmintic for use in parasitic gastro-enteritis, but in cattle there is need for something better. Riek and Keith in Australia have tested a large number of new compounds as anthelmintics in cattle and Gordon has investigated the same compounds for use in sheep.

Riek and Keith (1957a) examined the value of toluene as an anthelmintic for cattle. It was found that withholding food and water overnight increased anthelmintic efficiency and that premedication with sodium bicarbonate to close the oesphageal groove was necessary. At a dose rate of 10 ml. per 100 lb. body weight, high efficiency was reported against *Haemonchus placei*, *Bunostomum phlebotomum*, *Cooperia pectinata*, *C. punctata*, *Ostertagia ostertagi*, and possibly against *Trichostrongylus axei*. The drug was ineffective again *Oesophagostomum radiatum*. It was observed that animals in poor condition sometimes staggered and collapsed within sixty minutes of dosing. Recovery took place in three to four hours.

1:8 Dihydroxyanthraquinone at a dose rate of 2·5 grams per 100 lb. body weight was shown by Riek and Keith (1957b) to be highly effective against *Haemonchus placei*, *Cooperia* spp. and *Oesophagostomum radiatum* in calves and no untoward effects were reported. Gordon (1957b) investigated the value of several quinones as anthelmintics in sheep, but only 1:8 dihydroxy-anthraquinone had any useful anthelmintic effects. It was highly efficient against parasites of the large bowel but not very effective against other gastro-intestinal parasites.

Following their successful use as ascaricides in domestic animals, Gordon (1957a) investigated the use of various piperazine compounds in sheep and concluded they are useful only against *Oesophagostomum* spp. Marquardt and Fritts (1956) also concluded that piperazine compounds were ineffective against species of *Trichostrongylus*, *Nematodirus*, *Strongyloides* and *Trichuris* in sheep. Riek and Keith (1958a) reached similar conclusions about the efficiency of piperazine compounds in cattle although piperazine-1-carbodithioic betaine when injected directly into the abomasum was highly effective against *Haemonchus placei* and *Cooperia* spp.

Even more recently Riek and Keith (1958b) have investigated the value of certain organic phosphorus compounds as anthelmintics in cattle. 0,0-dimethyl 2, 2, 2-trichloro-1-hydroxymethyl phosphonate (Neguvon) proved to be highly effective against both the larval and adult forms of *Bunostomum phlebotomum*, *Cooperia* spp. and *Trichostrongylus axei* when given at a dose rate of 2 grams per 100 lb. body weight. Although no toxicity was observed in trials carried out in Queensland, toxic effects were seen in calves dosed in Victoria, although no deaths occurred. At Weybridge my colleague, J. F. Michel, has used Neguvon on six animals, five of which showed toxic effects and one of which died. In sheep, Gordon (1958b) showed Neguvon to be highly efficient against *Haemonchus contortus* in doses of 3 grams per 100 lb. body weight and to remove all these worms at 5 grams per 100 lb. body weight. The drug was effective only if swallowed directly into the abomasum. Variable results were obtained against *Oesophagostomum columbianum* even at dose levels toxic to some sheep. The compound 3-chloro-4-methyl-7-oxycoumarine diethyl thiophosphoric acid (Bayer L 21/199) was an effective anthelmintic but too toxic for general use, whilst 0,0-dimethyl-0-2, 4, 5-trichlorophenyl phosphorothioate (Dow E.T.57) showed no promise as an anthelmintic against gastro-intestinal worms in sheep. More work is required before these organo-phosphorus compounds can be confidently recommended for use in the field, and in cattle they must certainly be used under veterinary supervision with supplies of the antidotes atropine and 2-pyridine aldoxime methiodide at hand.

Although a great deal of work has been carried out in the last few years in an endeavour to find an anthelmintic superior to phenothiazine for gastro-intestinal parasites of sheep and cattle, nothing has yet been discovered which is an adequate substitute. In

certain special cases, for example cooperiasis in cattle, some of the newer compounds may be preferred.

In the treatment of nematodiriasis in lambs, however, a spectacular advance has been made in the discovery of the efficiency of bephenium embonate. Rawes and Scarnell (1958) have shown that at a dose rate of 250 mg. per kg. body weight this compound is highly effective against both adult and larval forms and its use in the field has supported their conclusions. The drug is non-toxic even when given at ten times the normal dose. Bephenium hydroxynaphthoate has also been reported to be highly effective against *Nematodirus* spp., Hignett (1959), and also against most of the other trichostrongylid nematodes of sheep. Gordon (1958c) also reports favourably on the action of bephenium hydroxynaphthoate against *Haemonchus contortus*, *Trichostrongylus axei* and *Oesophagostomum columbiamum*. More work is required before the value of this compound can be finally assessed, but it shows great promise as an anthelmintic in sheep.

When using anthelmintics in the treatment of parasitic gastro-enteritis it is important to remember that all members of the flock or herd will be infected and all must be treated whether they appear ill or not. Also, when using an anthelmintic such as phenothiazine which has no action on immature forms the treatment must be repeated once or twice at weekly intervals to eliminate worms which reach maturity subsequent to the first dose of the drug. Although the removal of the worms from a heavily parasitised animal is essential for its recovery, it may be so weak and debilitated as to require other treatment as well. This aspect of the treatment of worm disease is being considered by Mr. White in the next paper.

In addition to their use in the therapy of parasitic disease, anthelmintics can, of course, be used prophylactically. Gordon in Australia distinguishes between "strategic" treatments which are related to the seasonal trend of worm burden as revealed by epidemiological studies, and "tactical" treatments which depend on the recognition of the imminent increase of worm burden. Gordon (1958a), on the basis of extensive epidemiological studies, proposes a series of four treatments for the control of worms in the winter rainfall areas of Australia, whilst Banks (1958), working under different climatic conditions in South Australia, doubted the value of routine anthelmintic treatment and stated that certainly no more than one treatment at weaning time was required. These divergent views show clearly the influence of climate on the necessity for prophylactic treatment, and in a climate so variable as that of Britain it is difficult to devise a system of prophylactic treatment which is universally applicable. Taylor (1943) has shown how the worm burden of a lamb builds up during the spring and summer reaching a peak in the late summer round about weaning time. From this information and with a knowledge of the life cycle of the worms involved, it is clear that monthly treatment with anthelmintic throughout the summer would keep the infestation at a low level with consequent benefit to the lambs. The fact that infestation reaches a peak at weaning time suggests that treatment then is particularly important. Recommendations on these lines will be found in B.V.A. publications and in the advisory leaflets and bulletins of the Ministry of Agriculture.

The work of Harbour *et al.* (1946) and Pollard *et al.* (1949) provides evidence of the value of this type of prophylactic control, although Parnell *et al.* (1955) were unable to demonstrate any increase of weight gain by dosed sheep over controls, although the worm burden was reduced. The extent to which routine anthelmintic treatment is justified in the control of worms depends on the circumstances on each farm—the stocking rate, the grazing management and other factors may all exert a profound influence on the intensity of the worm problem. The veterinarian can give valuable advice to the farmer in devising a system of preventive treatment best suited to his particular circumstances.

The use of small daily doses of phenothiazine in the control of parasitic gastro-enteritis has been extensively used with great success in the U.S.A., but has never found favour in this country. Although the American workers believed ½-1 gram daily doses given in a salt lick actually diminished the worm burden of the treated sheep, work by the writer (Gibson, 1950) proved conclusively that the worm burden was unaffected, and that the ingested larvae could develop to maturity. It was shown, however, that the egg-laying capacity of the worms was reduced and that any eggs passed in the faeces failed to develop into infective larvae. The control of parasitic gastro-enteritis by low-level phenothiazine intake is achieved by reduction in pasture contamination and the reduction in pasture larval levels, and work carried out in this country by Harbour *et al.* (1946) and Pollard *et al.* (1949) has shown the value of this in the field. Leiper (1951) has also demonstrated the value of a combination of a full dose of phenothiazine prior to lambing following by small daily doses in the food for a period of nine weeks. Superior weight gains were recorded in treated lambs and blowfly strike was absent, whilst in the control group 25 per cent of the lambs were struck.

The possibility that the continued use of small daily doses of phenothiazine might result in the development of phenothiazine-resistant strains of nematode was recognised by Gibson (1949), and Drudge *et al.* (1957a, b) and Leland *et al.* (1957) have produced evidence that in a sheep flock which had received low-level phenothiazine for ten years the nematodes became resistant to the effects of low-level treatment. They were not, however, resistant to full therapeutic doses of the drug.

In America, where it is customary to feed salt to sheep, the phenothiazine can be conveniently administered as a 1:16 phenothiazine salt mixture. In this country, where regular salt administration is not practised, difficulties in administration of the drug arise and this may be responsible for the unpopularity of the system here.

References

BANKS, A. W. (1958). *Aust. vet. J.*, **34**, 20.
DRUDGE, J. H., LELAND, S. E., and WYANT, S. N. (1957) *Amer. J. vet. Res.*, **18**, 133.
——. (1957). *Amer. J. vet. Res.*, **18**, 317.
GIBSON, T. E. (1949). *Vet. Rec.*, **61**, 451.
——. (1950). *J. comp. Path.*, **60**, 117.
——. (1951). *Brit. vet. J.*, **107**, 377.

GORDON, H. McL. (1940). *J. Coun. sci. industr. Res. Aust.*, **13,** 85.
——. (1956). *Aust. vet. J.*, **32,** 258.
——. (1957a). *Aust. vet. J.*, **33,** 1.
——. (1957b). *Aust. vet. J.*, **33,** 39.
——. (1958a). *Aust. vet. J.*, **34,** 5.
——. (1958b). *Aust. vet. J.*, **34,** 104.
——. (1958c). *Aust. vet. J.*, **34,** 375.
HARBOUR, H. E., MORGAN, D. O., SLOAN, J. E. N., and RAYSKI, C. (1946). *J. comp. Path.*, **56,** 180.
HIGNETT, S. L. (1959). Private communication.
HUNGERFORD, I. G., and HOWE, K. (1944). *Agric. Gaz. N.S.W.*, **55,** 92.
KINGSBURY, P. A. (1958). *Vet. Rec.*, **70,** 523.
LEIPER, J. W. G. (1951). *Vet Rec.*, **63,** 885.
LELAND, S. E., DRUDGE, J. H., WYANT, S. N., and ELAM, A. W. (1957). *Amer. J. vet. Res.*, **18,** 851.
MARQUARDT, W. C., and FRITTS, D. H. (1956). *J. Amer. vet. med. Ass.*, **129,** 153.
PARNELL, I. W., DUNN, A. M., and MACKINTOSH, G. M. (1955). *Brit. vet. J.*, **111,** 195.
POLLARD, E. P., OWEN, R. W., and EVANS, G. D. (1949), *J. comp. Path.*, **59,** 54.
RAWES, D. A., and SCARNELL, J. (1958). *Vet. Rec.*, **70,** 251.
RIEK, R. F., and KEITH, R. K. (1957a). *Aust. vet. J.*, **33,** 162.
——. (1957b). *Aust. vet. J.*, **33,** 169.
——. (1958a). *Aust. vet. J.*, **34,** 1.
——. (1958b). *Aust. vet. J.*, **34,** 93.
ROBERTS, F. H. S. (1941). *Aust. vet. J.*, **17,** 130.
TAYLOR, E. L., and SANDERSON, K. M. (1940). *Vet. Rec.*, **52,** 655.
TAYLOR, E. L. (1953). *Vet. Rec.*, **55,** 117.
THOMAS, P. L., and ELLIOTT, D. C. (1957). *N.Z. vet. J.*, **5,** 66.
WARWICK, B. L., TURK, R. D., and BERRY, R. O. (1946). *J. Amer. vet. med. Ass.*, **108,** 41.
WHITTEN, L. K., CLARE, N. T., and FILMER, D. B. (1946). *Nature Lond.*, **157,** 132.
——, and FILMER, D. B. (1947). *Aust. vet. J.*, **23,** 336.
WHITTEN, L. K. (1956). *N.Z. vet. J.*, **4,** 63.

The Treatment of the Debility Associated with Severe Parasitism

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SUMMARY—While there have been, in the last two decades, very great advances in the science of parasitology, these have in the main been directed towards control of the parasite. Comparatively little attention has been devoted to the treatment of the debility which almost always accompanies parasitism.

It is this bodily wasting which accounts for the major loss to the farmer, and it is felt that the present methods commonly employed could, in some cases, be improved.

The paper therefore reviews the ways in which the debility is brought about, and changes which are involved, particular emphasis being given to the condition of anaemia which is usually present. This subject is discussed in some detail, mention being made of the different views held and conclusions made by research workers in this field.

The evidence in general suggests that anaemia is due in most instances to a direct loss of blood from host to parasite, though other factors which may influence it are: (a) lack of appetite; (b) poor food absorption; and (c) lowered efficiency of haemopoietic system.

With regard to treatment, some routine measures of husbandry are outlined for use in groups of animals which are the subject of parasitic disease. These are followed by suggested lines of treatment in a few chosen diseases, with some discussion as to the best methods of treating the complicating anaemia.

SEVERE losses from parasitic infestation are among the most ancient hazards of farming, and these losses can on occasion be of a magnitude equal to those of bacterial or viral origin, while their effects can be almost as remorseless as those of gamma radiation.

There can be few tasks so depressing to the general practitioner as the treatment of heavily parasitised young animals. It is with these thoughts in mind that the author has attempted to review the etiology and treatment of the severe debility associated with parasitism.

Too often one is tempted to confine one's attention to the elimination of the parasite without giving adequate thought to the treatment of the debility. It is this debility, with its associated anaemia, that is the cause of death in the majority of fatal cases rather than any tissue damage which the parasite may inflict upon the host, though there are exceptions in which the tissue damage is sufficient to prove fatal.

Thus, in acute fluke diseases death may result from the extreme injury suffered by the liver during the migration of the immature flukes, while some workers consider that the local injury suffered in severe cases of parasitic gastro-enteritis may in some cases cause death. One is also familiar with the case of the young pig in which the bile ducts are completely occluded by ascarids. In the main, however, death follows the profound anaemia and gross interference with nutrition which is the usual sequel to severe parasitic infestation.

In some instances where the intake of parasites has been heavy, within a short space of time the host will show signs very similar to shock, particularly in the young, highly susceptible animal. In these cases it is hard to believe the extent to which animals can deteriorate within so short a period of time as one week unless it is personally observed. This is well illustrated in the case of foals and calves suffering from acute strongylosis.

Thus it has been shown by Gibson (1954; 1955) that lambs can withstand a dose of larvae two or even three times a fatal dose, provided the period of intake is spread over several weeks.

It is also a well-known fact that the level of nutrition at the time of infestation can determine the outcome; thus animals on a high plane of nutrition can withstand higher doses of larvae than those on a low plane. This may simply be direct competition between parasite and host for the available food, but there is evidence to show that it relates to essential food factors such as vitamin B_{12} and trace elements, which are necessary for both host and parasite. It is thought that some of these "specific nutritive factors" may play an essential role in erythropoiesis, and in this connection Archer and Poynter (1957) working with ponies demonstrated an erythrocytopenia and erythroid hypoplasia which they considered might have arisen from a shortage of vitamin B_{12}, the available supply being absorbed by the parasites within the lumen of the gut.

In acute parasitic gastro-enteritis of the ruminant there is a marked decrease in appetite which is probably related to the pain and discomfort associated with acute abomasitis. This not only diminishes food intake, but also interferes with food absorption owing to the inflamed state of the mucosa.

Gibson (1957) was able to show that healthy lambs could make a greater liveweight gain on the same quantity of food as was being eaten by infected lambs, and he went on to show that, all things being equal, the infected lambs could never catch up in weight gain with the non-infected lambs. This apparent stunting effect has often been attributed to the presence of a toxin produced by the parasite, but so far this has not been conclusively proved.

Of all the pathological changes affecting the host, the most obvious and the most serious is the anaemia which occurs in so many different kinds of parasitic infestation. It is most noticeable in strongylosis affecting foals, calves or lambs; it is equally well recognised in fluke disease and less often with ectoparasites, though the occasional death of a dog as the result of a heavy burden of blood-sucking lice may be seen.

The anaemia may be produced in a variety of ways, the most usual being due to a straightforward withdrawal of blood by the parasite from the host. In this respect allowance must be made for the blood which leaks from the wounds inflicted by the parasite in the wall of the intestine. This may be greater than one would anticipate due to the presence of anticoagulens secreted by the worm. Andrews (1942) showed that a lamb with severe haemonchosis could lose 6 oz. of blood a day, while it has been shown that a dog carrying 200 ancylostomes may lose as much as 5 oz. a day.

Richard, Shumard and Pope (1954) estimated that over a five-week period a lamb could lose 3·2 times its original blood volume.

It is not only by direct bleeding, however, that parasites can cause anaemia, since some of them, such as *Trichostrongylus axei*, do not appear to suck blood but to interfere with the absorption of essential nutrients to such an extent as to inhibit normal blood replacement. It would also appear that this interference with erythropoiesis may result in the production of erythrocytes of shorter life than the normal, thus aggravating the anaemia.

In most acute infestations the first signs of anaemia are seen from six to ten days after infection and death may occur before the worms commence egg-laying.

If, however, the host can survive the first four to five weeks, the daily blood loss tends to become stabilised as the haemopoietic system gears itself to the increased daily loss. This was shown by Richard, Shumard and Pope, who bled non-infected control lambs, removing daily a quantity of blood equal to that which they estimated was being lost by similar lambs infected with 40,000 *H. contortus*. It must be appreciated that the iron of the haemoglobin is completely lost to the host in this type of anaemia, there being no breakdown product remaining for resynthesis to haemoglobin, so that any replacement iron must be taken in with the diet once the body reserves are exhausted.

This may partly explain why parasitism produces such profound debility in young animals or in pregnant females, such as sows affected with *Hyostrongylus rubidus*, where there is a heavy burden of growth as well as body maintenance.

This daily loss of blood does not only bring about a drop in the red cell count, but also results in a considerable lowering of serum globulin and this, in turn, favours the onset of oedema which is a common feature with many parasitic diseases.

The most noticeable effect on the leucocyte system is an eosinophilia at the site of worm development. Thus Archer and Poynter (1957) showed marked eosinophile infiltration of the intestinal wall in young ponies affected with nematodirus, and Mackenzie (1959) showed marked eosinophile infiltration into the lung tissue during the course of lungworm infection in pigs. There was also a general eosinophilia of peripheral blood in both cases.

It should be recalled that, in addition to causing digestive disturbance and anaemia, parasites may give rise to debility in a number of other ways. Thus continued loss of rest as is seen in sarcoptic mange and sheep scab is sufficient to account for loss of condition, while the severe cough of lungworm infection may in part explain the wasting associated with this condition particularly in the adult cow, although the systemic effect of the bronchopneumonia must probably be the major cause.

Treatment

It may be considered that the preceding discussion on debility of parasitism is too long, but it is felt that no logical approach can be made towards the treatment of this condition without a reasonable understanding of the pathological changes which take place in the host.

When one comes to consider the treatment of this debility, it is quickly apparent that each case should be given individual consideration owing to the very large number of variable factors associated with its incidence; and yet in some species the question of cost quickly rules out the provision of expensive techniques which would never provide a financial return, and in these cases one can only adopt a treatment on general lines.

In this paper it is assumed that appropriate antiparasitic treatment has been instituted, but it could well be emphasised that this must also be maintained if success is to be hoped for, since with so many parasites the larval forms are not susceptible or are inaccessible to the action of the drug and can only be dealt with when they become adult. In addition, there are very few drugs which approach 100 per cent. efficiency with one dose.

In all outbreaks of parasitism it is necessary to make a general appraisal of the situation, including an estimate of the reasons why clinical disease has arisen, and a plan of husbandry to restore the animals to health as economically as possible.

In order to do this the following facts are required:

1. The clinical condition of the affected animals, including knowledge of their age and type.

2. How long clinical disease has been in evidence, and whether it would appear to be a gradual or sudden severe infestation.

3. Where possible, an accurate identification of the species of parasites involved.

4. How many animals there are running together and whether they are of approximately the same age and size.

5. A survey of the pasture available for this stock for the next two months, together with a rough estimate of its probable nutritive value and parasitic status, these being largely dependent on the season of the year at which disease occurs.

6. The availability of supplementary food.

Where the clinical picture is not too severe and the infection does not appear to have been heavy and concentrated, then the stock is probably as well left

out at grass if they can be kept moving forward on to good clean pasturage.

It is most essential in young animals to provide high quality supplementary food containing, if possible, such items as yeast, linseed or animal protein.

The need for this must be firmly maintained even in the face of opposition from the owner. In this respect it is interesting to note the work of Richard, Shumard and Pope (1954) who, when working on parasitic gastro-enteritis of lambs, found marked benefit from the feeding of mineral mixtures containing salts of sodium, iron, cobalt, copper, iodine and manganese with steamed bone meal.

It has further been stated that the full benefits of any treatment of anaemia can only be attained together with an adequate diet of good quality. A most important practical point to bear in mind is that groups receiving supplementary food should be of approximately the same age and weight and with plenty of trough space in order to ensure that all have an equal share.

The above constitute the general measures one can usually adopt particularly with animals of smaller economic value such as sheep, though in the larger animals various other steps can be employed.

When treating calves affected with parasitic gastritis in which diarrhoea is severe, it is usually preferable to house them and an effort should be made to control the diarrhoea by means of normal intestinal astringents. A course of sulphadimidine for three days will often help in this respect, presumably by the control of intercurrent bacterial infection. Where dehydration is very marked saline may be administered subcutaneously.

It is difficult to make any set rule regarding the treatment of young cattle affected with parasitic bronchitis. In those exceeding fifteen months there seems little advantage in housing them, provided they are willing to feed, and can be kept in reasonably dry pasture where they must receive a concentrate supplement. With younger stock that will in any case be housed later in the year it is better to yard them at once, picking out any which show signs of pneumonia for individual treatment.

In dealing with the debility of husk, it is difficult to divorce one's treatment from the treatment of the husk itself, and I feel one must consider the employment of one of the chemotherapeutic agents at present advocated and about whose usefulness there is such divergence of opinion. Where bronchopneumonia exists the use of antibiotics and cardiac stimulants will help to cover the critical period, while the use of oxygen may be considered in valuable animals. When housing this stock undue crowding must be avoided, as this seems to increase the spread of bacterial pneumonia.

In the case of foals affected with severe strongylosis, housing and good nursing are essential, and where diarrhoea is troublesome benefit may be derived from some of the milder astringents.

Injections of vitamin B_{12} may be employed with advantage to assist in promoting appetite, while some of the injectable forms of iron can be used to assist in combating the anaemia. There are also certain tonic preparations of mineral and vitamins blended with food into a palatable base which can be useful in promoting recovery in an animal where the forcible administration of medicine is to be deprecated.

Anaemia

It is interesting to notice the extreme paucity of information regarding the treatment of anaemia in animals other than the anaemia of the young pig.

The old text-books carried many more prescriptions containing salts of iron than are used today, probably because the lack of specific anthelmintics made treatment of the resultant debility the only course of treatment available to them. It is clear that the absorption and utilisation of iron is not at all well understood and that better results can sometimes be obtained where iron is given together with other agents such as copper, cobalt and vitamins, particularly those of the vitamin B complex. It is also necessary that the level of nutrition must be reasonably good if the greatest absorption is to be realised.

The salt most commonly employed by the mouth in both human and veterinary medicine is iron ammonium citrate. American workers treating hook worm disease in the human (Rhoads, 1934) were able to demonstrate very marked improvement in the anaemia by using this preparation of iron, even where no anthelmintic treatment was given, and they considered it preferable to improve the anaemia before commencing to employ anthelmintics. There are now several forms of iron which are injectable and which do provide a helpful contribution to this problem.

In many cases, however, one would prefer a more immediate means of restoring the blood supply to normal levels and here the question of direct blood transfusion must come to mind. So far this is in the very early stages in the veterinary field, partly owing to lack of knowledge with regard to blood groups in our patients as well as the risk of carrying latent disease from one animal to another. Some workers have already used blood transfusions in dogs to help in surgery, while others are finding them helpful in combating the profound anaemia associated with piroplasmosis. In these cases workers are prepared to face the risks of incompatability where the patient will almost certainly die unless immediate relief can be given.

One does, however, feel that it would be a big step forward if transfusions could be more often employed, since the donor can usually be provided from the same farm, thus reducing the chance of spreading some other latent infections.

It is well appreciated that this account has been written from experience with parasitic infections as seen in one part of the country and that there may be considerable divergence of approach under different farming conditions. The ventilation of different viewpoints as a result of this must lead to a better understanding of the problem as a whole.

References

ANDREWS, J. S. (1942). *J. Agric. Res.*, **65**, 1.
ARCHER, R. K., and POYNTER, D. (1957). *J. Comp. Path.*, **67**, 196.

BELDING, D. L., Clinical Parasitology. 2nd ed. D. Appleton-Century Co., 1952.
GIBSON, T. E. (1954). *J. Comp. Path.*, **64,** 127 and 360.
——. (1955). Ibid., **65,** 143 and 317.
GRUNSELL, C. S. (1955). *J. Comp. Path.*, **65,** 93.
LAPAGE, G. *Veterinary Parasitology*. Edinburgh. Oliver & Boyd, 1956.
MACKENZIE, A. (1959). *Vet. Rec.*, **71,** 209.
MARTIN, C. J., and ROSS, I. C. (1934). *J. Helminth.*, **12,** 137.
RHOADS, C. P., *et al.* (1934). *Medicine*, **13,** 317.
RICHARD, R. M., *et al.* (1954). *J. Anim. Sci.*, **13,** 274.
SPEDDING, C. R. W. (1954). *J. Comp. Path.*, **64,** 93.
TAYLOR, E. L. (1934). *J. Helminth.*, **12,** 143.

THE SPEAKERS' INTRODUCTION

The Chairman, Mr. D. F. Oliver, said that it was his pleasure to introduce two speakers on subjects which he was sure would be of equal interest to clinician and research worker alike.

First, he would call upon Mr. Gibson, and then he would ask Mr. White to present the second paper.

Mr. T. E. Gibson, in presenting his paper, said that as it had been circulated in advance there was no need for him to read it in detail, but perhaps it would be helpful to reiterate some of the main points to serve as a basis for discussion.

At the beginning of the paper it was pointed out that parasitic gastro-enteritis was not a single disease but a complex of diseases caused by some 6 or 8 species of parasitic worm and in consequence the ideal anthelmintic was one with a wide range of activity coupled with complete lack of toxicity for the host. This goal had not yet been achieved but phenothiazine, introduced two decades ago, came as near as we had yet got to this ideal. Phenothiazine had many deficiencies which were discussed in the paper.

Particular attention had been paid recently to the influence of particle size on the efficacy of phenothiazine preparations and since the paper had been prepared one or two other pieces of work had come to his notice. Douglas *et al* (1959) have demonstrated a linear relationship between specific surface area and anthelmintic efficiency within the approximate range of 5,000 to 25,000 sq. cm. per gram. Preparations having a mean particle diameter of 1μ are 98 per cent. efficient, but when the average particle diameter reaches 10μ the efficiency falls as low as 63 per cent.

In addition to the question of particle size which was discussed more fully in the main paper the influence of purity on the anthelmintic activity of phenothiazine has been investigated recently by Douglas *et al* (1959) and Baker *et al* (1959). It was found that a pure phenothiazine preparation with an average particle diameter of 4·8μ was an efficient as the American National Formulary phenothiazine with an average particle diameter of 1·9μ. On the basis of these observations it is suggested that for sheep the ideal preparation of pure phenothiazine would have a specific surface area of 6,000-7,000 square centimetres per gram or an average particle diameter of 6μ or less whilst the N. F. phenothiazine must have a specific surface area of 25,000 square centimetres per gram or an average particle diameter of 2μ or less.

Forsyth (1959) in a recent letter in the *Australian Veterinary Journal* had pointed out that he had been working for some time on the influence of both particle size and purity on the anthelmintic efficiency of phenothiazine. He reported that when purity fell below 70 per cent. anthelmintic action became extremely erratic and at 85 per cent. purity phenothiazine was 80 per cent. efficient. He defined the minimum qualifications of a phenothiazine preparation as being 85 per cent. purity and a specific surface area of 12,000 square centimeters per gram; a definition not unlike that given by Baker *et al* (1959).

The indications were, therefore, that phenothiazine for use on the field must be pure and probably more finely ground than had been the custom. This matter was still under investigation and future work would clarify the position, but these factors might explain some of the erratic results obtained with phenothiazine in the past.

One aspect of the use of finely ground phenothiazine which should not be overlooked was the possibility of increased toxicity arising through greater absorption. The hazard was greater in cattle than in sheep which appeared to be unaffected by massive doses of phenothiazine. No reports had been received from the field of increased toxicity when finely ground preparations had been used but the matter was being kept under observation. The only indication had been from the United States where Richardson and Todd (1958) had reported a deeper colouration of cow's milk following the administration of micronised phenothiazine than when courser particles were used.

Turning to the newer compounds he considered firstly the organic phosphorus compounds and secondly the bephenium compounds. Three of the organic phosphorus compounds had been used fairly extensively and some of these compounds possessed useful athelmintic activity agains the gastro intestinal nematodes of cattles and sheep. There was considerable risk to toxicity with the most active compounds which precluded their use in general practice. Practitioners may decide to use them in instances where disease was caused by particular worms against which these compounds were active when phenothiazine failed. They must, however, be prepared to watch the animals for symptoms of toxicity after the drug had been administered.

The bephenium compounds had revolutionised the treatment of nematodiriasis and this group of compounds were particularly interesting since they were extremely active against immature worms. In addition to their use in therapy they could be used in the prevention of nematodiriasis. A recent paper by Rawes and Scarnell (1959) had shown that 3 doses at 3-weekly intervals commencing at the beginning of May were effective in preventing the disease and work carried out by his colleague W. V. B. Sinclair of Leeds had showed similar results.

He proposed not to repeat details of the treatment of parasitic gastro-enteritis and of its control and prevention dealt with in the main paper but wished to conclude by saying a few words about the administration of anthelmintics, in particular the use of dosing guns. Slides were shown illustrating 3 forms of dosing gun and their use was explained. The first gun was a simple dosing pistol having interchangeable nozzles for cattle, adult sheep and lambs and was useful for dosing small numbers of animals and when using an anthelmintic which would not readily stay in suspension. The other two were forms of automatic drenching gun for sheep and the second which incorporated the McMaster oesophogeal tube was considered to be the most elegant instrument at present available for drenching sheep.

References

BAKER, N. F., ALLEN, P. L., LONGHURST, W. M., and DOUGLAS, J. R. (1959), *Amer. J. vet. Res.*, **20,** 409.
DOUGLAS, J. R., BAKER, N. F., and LONGHURST W. M. (1959), Ibid., **20,** 201.
FORSYTH, B. A. (1959). *Aust. Vet. J.* **35,** 99.
RICHARDSON, T., and TODD, A. C. (1958). *Amer. J. vet. Res.* **19,** 610.
SCARNELL, J., and RAWES, P. A. (1959). *Vet. Rec.* **71,** 163.

The Chairman said that the author of the next paper, Mr. White, had shown particular interest in the disease conditions of cattle and sheep. He had been a member of the Technical Development Committee for about ten years and for the past year he had been its valued Chairman.

Mr. J. B. White, presenting his paper, said that there must be many among those present who, on reading the paper, felt that it amply justified the old saying that fools rush in where angels fear to tread. At least, that was the way he had felt ever since he had agreed to write it! However, as a practitioner he would be satisfied if he could at least stimulate his audience to recount the many ways in which they attacked this problem of debility associated with parasitism. He hoped that as he was a practitioner they would not hesitate as much as they might if he were an expert. Practitioners had a great wealth of clinical knowledge which would be of tremendous service if only it could be drawn upon.

When he came to write the paper he was faced with the problem: just what is this debility with which everyone is familiar? They knew very well that with present-day anthelmintics one could eliminate a very large percentage of the parasites which affected animals. Yet, in a great many instances, these animals were still doomed to die. The problems of last autumn brought this forcibly to his mind. Then one saw animals which were practically walking skeletons, but nothing on earth that one could do seemed to be able to save them. Surely some means must be available to improve the position.

When he looked into the literature on this subject he found that there was very little, particularly on the subject of anaemia in animals other than the suckling pig. This condition of anaemia was brought about in a variety of ways which he had outlined in the paper, the main one being the withdrawal of blood from the host to the parasite. Mr. Gibson had shown that the parasite *T. axei*, was not a prodigious blood sucker itself, and yet it produced a very severe anaemia. He did not think that even now the way in which it did it was very clear. It might be that it absorbed certain esential nutriments which the host required but which the worm took. It also produced a condition of inflammation throughout the alimentary tract or throughout the stomach and small intestine which could severely interfere with the normal absorption of food. It also caused a considerable degree of discomfort to the animal, sufficient probably to account for the lack of appetite which was a noticeable feature in parasitism.

Until one studied the literature he did not think one had any conception of the amount of blood that was withdrawn from these animals. The quantities taken were quite striking. In addition to the anaemia and interference with food intake and absorption, there were other causes of debility including tissue damage such as one saw in fluke disease or the damage one encountered in husk, associated in that case with bacterial interference as well. Also, there were those conditions which were associated with loss of rest such as sheep scab. Therefore, there was quite a number of ways in which debility was caused.

The dearth of literature on the subject was quite extraordinary. He had found a certain amount of literature on hookworm disease in the human subject, and saw that doctors relied basically on the administration of iron ammonium citrate. It was interesting to note that in the South Seas it was found preferable in many cases to treat the debility with iron before starting the administration of anthelmintics. Better results were achieved by trying to build up the host to some extent before attacking the parasite with anthelmintics.

When one went on to a farm with a parasite problem he thought that too often one was tempted to diagnose a condition of parasitic gastro-enteritis and to say, "All right. We will dose this animal with phenothiazine" and then to hope for the best. In his view that was where they fell down too often. It was necessary to size up the problem and to get the over-all picture. There were many things to consider: the age of the animal, the intensity of infestation, for how long it had been infested and the number of animals it was with. It was important to know whether the animals were of similar size and whether the ones that were suffering were the underdogs that were being deprived of the proper food supply which they should get. That was an important point for them, as clinicians, to remember. Especially where farms were large and 60 or 70 animals were together, it was absolutely essential if parasitism arose that the animals should be split up into relative sizes so that those which needed any extra food could get it. That was a fundamental point. As veterinarians they must drive that home to the farmer or he would never do it.

Since the paper had been written early in the year, the parasitic diseases handbook had been circulated and it contained a description of the means by which blood transfusions could be used. He had read Dr. Hall's paper "Blood grouping in domestic animals" and he noted that Dr. Hall was still fairly cautious in his approach to the question of blood transfusions in animals. Apparently a single transfusion was fairly safe, but Dr. Hall was very cautious about the application of further transfusions. Also, one should bear in mind the problem of the possible transfer of disease. If a transfusion was done on a farm which was self-contained, then probably the risk was not great because the animal that was being given the blood would probably be exposed to the same infections as the animal from which the blood was taken.

He would be most interested to receive more information on the subject.

The Opener

Mr. G. S. Peyton, the opener of the discussion, thanked the authors, Mr. Gibson and Mr. White, for the time and trouble they had taken in preparing their papers, both of which were excellent. Obviously, they were masters of their subjects, and the Association was extremely lucky to have received the benefit of their knowledge.

Parasitic gastro-enteritis and the debilities arising therefrom were obviously of very great economic importance. They were of great importance to the farmer and to the practising veterinary surgeon. No doubt many present would have had a great deal to do with the treatment of these conditions, but there were one or two points mentioned by the authors which he wished to emphasise. One was the extreme rapidity with which a sudden and heavy infestation could cause great damage. He had known a flock of about 400 lambs in the charge of a very good and experienced shepherd lose 30 lambs in the space of 2 days with a heavy Nematodirus infestation. The other feature he wished to stress was that of weather conditions.

In the south-east in some years parasitic gastro-enteritis caused relatively minor trouble, but in other years it caused a lot of trouble. The over-riding feature there was that of weather conditions. In that part of the country most stock-owners carried out routine dosing of sheep and young cattle, and they were of the opinion that they got better results where they alternated dosing with phenothiazine and a copper and nicotine mixture than if they stuck solely to phenothiazine. He would like to know whether there was anything in that or whether it was just their imagination.

Mr. Gibson's remarks on the size of the particles were most interesting and enlightening. He had intended to ask whether size of particle had any bearing on toxicity, but Mr. Gibson had answered that question in advance. There was a great deal of controversy, at least among stock owners, as to whether when using phenothiazine it should be given in the form of a drench or as a tablet. The tablet had the great advantage of causing less discoloration of the fleece. He wondered whether Mr. Gibson had any views on the efficiency of the respective methods.

Mr. Gibson had reviewed the use of a number of other anthelmintics. From the point of view of a practising veterinary surgeon, he would say that the work done by Scarnell and Hignett had been of the greatest benefit. They felt now that they could keep Nematodirus more or less under complete control.

Personally, he was not nearly so happy with phenothiazine. They seemed to get variable results which might be explained by what Mr. Gibson had had to say. Nevertheless, one looked forward to the day when they would have something of greater efficiency which did not suffer from the defects of the newer drugs about which they had been told. He wondered whether in the foreseeable future they were likely to have a drug for the treatment of parasitic gastro-enteritis which could be administered by injection. He also wondered whether they were ever likely to see an efficient vaccine.

Mr. Gibson's remarks about dosing sheep were most interesting to him. He recalled that last autumn a flock of 400 lambs were dosed with phenothiazine. In the subsequent week or so 12 of them died and on *post-mortem* examination all were found to have gangrenous wounds in the throat and oesophagus, and it was fairly definite that the dosing had caused their death.

He thought that Mr. White had been extremely brave to write a paper on such a subject. He endorsed every word said by Mr. White about the difficulty of finding anything in print that was of much help. The paper went fully into the question of anaemia and in his remarks Mr. White had enlarged on the other factors. To him the major problem with

a really debilitated animal was in getting it to feed. He wondered whether Mr. White had any other suggestions to make about that in addition to those he had made already. Like Mr. White, he would be extremely pleased if anyone present would give details of first-hand experience with blood transfusions. That appeared to be the obvious way of dealing with anaemia, but so far most of them seemed to fight shy of it.

Before closing, he wished to congratulate Mr. Gibson and Mr. White on the excellence of their papers.

The General Discussion

Mr. W. T. Harrow (Wilmslow): "Mr. Gibson has referred to his earlier paper, published in 1951, in which he was able to detect no difference in activity between several samples of phenothiazine of varying particle size, and he has said that this was because even his coarsest sample was fine enough to be highly efficient.

"I have recently re-read that paper and am afraid that I cannot agree with this explanation. The finest sample which Mr. Gibson used on those experiments was 100 per cent. effective against *Trichostrongylus axei*, and the 'bulk of the mass' of that sample had a particle diameter of 40 to 120 microns. The 'bulk of the mass' means 50 to 60 per cent. by weight, so that, in more recent parlance, not more than 50 per cent. of the particles in those samples were less than 40 microns in diameter. This is certainly not fine particle material. It is, in fact, extremely coarse. It must be remembered, however, that Mr. Gibson's material was at least 95 per cent. pure, by chromatographic analysis, whereas we now know that Gordon's was probably not more than 70 per cent. pure. Mr. Gibson has mentioned the work of Douglas and Baker in America and also that of my colleague Forsyth in Australia on the effect of purity on the efficiency of phenothiazine. I believe that it was the purity of his samples which was responsible for the results which he obtained in his earlier work. Further work needs to be done, but I believe that it will be found that the purity is every bit as important as the particle size of phenothiazine in determining its efficiency."

Miss O. Uvarov (Greenford): "I should like to thank the speakers for their papers and to suggest that more work should be done on the treatment of debility and dehydration following parasitic infection. In addition to the use of saline in cases of dehydration, has Mr. White used blood volume expanders or plasma substitutes in such cases? Clinical reports indicate that some of these substances can produce good results. It may also be important to consider the endocrine system in a debilitated animal. The work of Selye *et al.* has clearly shown that exhaustion of the adrenal follows exposure to stress. If the theory of 'general adaptation syndrome' is accepted it is feasible to suppose that in the debilitated animal the 'stage of exhaustion' may be present. In practice it has been shown that in some such cases the use of one of the corticosteroids, in addition to the more specific therapy, can produce good clinical results. It is difficult to run controlled field trials with corticosteroids in such diseases, but one accepts the findings of clinicians that corticosteroids do produce good results in cases where otherwise recovery appears to be doubtful.

"The use of corticosteroids in infections and some cases of shock in man have been reported. A similar field of treatment exists in veterinary practice, provided that the uses of these substances is safeguarded by adjunct therapy with antibiotics, or other drugs. Have the speakers had any experience with the use of corticosteroids in similar conditions in animals?"

The Replies

Mr. T. E. Gibson, in reply to the discussion, said he wished first to deal with the question of alternate dosing with phenothiazine and copper sulphate—nicotine sulphate mixture which had been mentioned by several speakers. There was no theoretical reason why this should be more beneficial than the use of phenothiazine alone but he would not wish to discourage those who considered it beneficial from continuing to use the alternate system.

The question of the administration of phenothiazine by drenching as against tablets had been raised by several questioners. In his view drenching was to be preferred to the use of tablets. In his own flock of experimental sheep he had originally used tablets feeling that more accurate dosage could be achieved. Experience had shown however, that the administration of tablets was time consuming and frequently accuracy of dosage was vitiated by spitting out of tablets which had apparently been properly administered. After turning to drenches and having used the three types of gun described earlier he had now concluded that the best way of administering phenothiazine was by means of the gun incorporating the McMaster oesophageal tube. The method was quick and accurate and resulted in no trauma whatsoever. The tube went down the oesophagus without any trouble at all. There was no difficulty in keeping the phenothiazine in suspension in the rubber bag carried on the operator's back. The phenothiazine sold nowadays kept in suspension readily and in addition the bag could be squeezed periodically to stir up the mixture.

Mr. Peyton had said a more efficient drug than phenothiazine was required. It was quite true that it was by no means ideal but was the best drug yet discovered for parasitic gastro-enteritis. Pharmaceutical workers all over the world were screening compounds as fast as they could in the hope of finding something better but had not yet been successful.

Mr. Peyton had also asked about vaccines. Investigations were at present being carried on the development of a vaccine against certain gastro intestinal nematodes of sheep. More work would have to be done, including field trials, before the value of the product could be finally assessed.

He had nothing to add on the question of the toxicity of small particle size phenothiazine raised by Mr. Harrow. Mr. Harrow had suggested that the phenothiazine used in his (Mr. Gibson's) experiments some years ago was coarse by modern standards and that his good results were referable to the purity of the samples used. Mr. Gibson regretted being unable to remember the exact figures for particle size quoted in his paper and had no information as to the purity of his samples. He felt that as far as he could remember the samples tended to be fine rather than coarse but it would probably be well to re-read the paper in the light of what Mr. Harrow had said.

Mr. Leiper and some others had said that the intestinal parasites were not removed even by regular doses of phenothiazine. Although phenothiazine was not so active against parasites of the small intestine as against those in the abomasum he felt that increasing the dose would help to overcome that deficiency.

Dr. Montgomerie had raised the question of a chronic enteritis resulting from parasitic infestation. Work carried out some years ago with *Trichostrongylus axei* had shown there to be three phases in the course of the disease. The first was an acute phase followed by a subacute phase followed lastly by a chronic phase. The subacute stage was characterised by ulceration of the abomasal mucous membrane which in the chronic phase had healed and the animal appeared clinically normal.

Mr. Cook had said that inadequate doses of phenothiazine were recommended by the manufacturers. Mr. Gibson agreed with this and said his calculations failed to make the dose recommended by manufacturers more than about 25 grams for adult sheep whereas not less than 30 grams should be given. When phenothiazine was first introduced *Haemonchus contortus* was much more common and as this parasite is easily removed with phenothiazine the manufacturers reduced the dose of what was then considered an expensive drug. Mr. Gibson did not think different doses of phenothiazine were required in different parts of the country unless it could be shown that the predominant parasites were different.

Mr. J. B. White, in reply to the discussion, said that Mr. Gibson had dealt adequately with many of the questions. He was strongly in favour of alternate dosing with anthelmintics. He did not necessarily confine himself to phenothiazine and

copper and nicotine. He had a certain liking for tetrachlorethylene, although that did not seem to find a great deal of favour in this country. He wondered whether any of the value of the copper and nicotine was in the copper, not as an anthelmintic at all but as a help in the anaemia problem which went with this disease.

Mr. Peyton had asked whether there were any other means of treating animals for lack of appetite. All he would say was that in some cases, particularly in calves where the scouring was very bad, they had found the use of sulphadimidine for 2 or 3 days to be quite helpful in checking the scouring and giving the animal a fresh start, in which case its appetite improved. That might also have some relation to what was said by Dr. Montgomerie. Those who had seen calves reared by artificial means—milk powders and such like—knew that they also could suffer from chronic enteritis. There were features in these cases that were not unlike those in chronic parasite cases. Dr. Montgomerie was probably quite right in thinking that chronic enteritis was a big factor.

The question of photosensitisation had been discussed. With the finer types of phenothiazine they must bear this in mind when they came to calf dosing. There had been talk of stepping up the dose. That must be related to sheep and they must be very careful when dealing with calves. One had to be most cautious about stepping up the dose when dealing with calves.

There was another danger with the finer phenothiazine and that was not to the animals but to the folk who were handling the substance. He gathered that the makers would only put out the very finest forms in a suspension because of the danger. It was such a fine dust that it was most difficult to control.

He had been most interested in the 50 cases of blood transfusions which had been mentioned, and he hoped that they would be written up because anyone who had had so much experience had a lot to offer to the practitioner of the country.

One or two people had mentioned the increasing trouble they experienced with Trichostrongylus and the poor results obtained with phenothiazine. He thought that routine dosing was a definite mistake. If they could avoid the difficulty by better husbandry they should do so. Also, while on the subject of increasing the dosage of phenothiazine, he thought that one could well afford to increase the frequency of dosing rather than of necessity increasing the dose.

There was one final point with regard to blood transfusions. His own natural caution went back to their days at the Royal Veterinary College when they were being taught about tropical diseases by the late Dr. A. L. Sheather. He recalled Dr. Sheather saying that in those days he had tried transfusions, with results rather similar to those mentioned by their colleague who had spoken about mules.

A vote of thanks to the authors, proposed by the Chairman, was carried by acclamation, and the session then terminated.

Diseases of Mink

BY

K. W. HEAD

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SUMMARY—A survey of 896 mink autopsies performed at the Royal (Dick) School of Veterinary Studies in the last ten years forms the basis for this review of disease as it occurs in mink kept on British fur farms. Errors in diet accounted for one-fifth of these cases. Generalised specific infections were responsible for a further fifth of the deaths (distemper caused one-tenth of these losses; salmonellosis, tuberculosis and fusiformis infections being the other common diseases in this group). Diseases of the alimentary tract caused a further tenth of the losses, non-specific enteritis and penetrating foreign bodies being the main sub-groups considered under this heading. Diseases of the respiratory system (mainly pneumonia and pleurisy) and of the urogenital system (urinary calculi and metritis) were each responsible for just under one-tenth of the cases. The Aleutian mink disease complex and poisoning (both food poisoning and chemical poisoning) also each caused slightly less than one-tenth of the deaths. The other disease conditions encountered are briefly mentioned.

Some attempt has been made to correlate the incidence of the more important diseases with farm management and to show how the disease picture as a whole has changed with the growth of the mink industry in the U.K.

Introduction

THE mink, *Mustela vison*, belongs to the Mustelidae, other members of the genus being the ferret, polecat, stoat and weasel. Some idea of the growth of the mink farming industry in the United Kingdom can be gained from Table I, which also includes the colour phase distribution figures demonstrating the rapid rise in numbers of mutation mink in response to market demand. Table II gives an indication of the annual distribution of the major diseases. It will be appreciated from this table that certain diseases have increased in frequency as these mutant colour phase mink have increased in number. This may indicate a colour phase predisposition to these diseases.

The paper describes in brief the findings in 896 mink autopsies performed at the Royal (Dick) School of Veterinary Studies between 1st January, 1949, and 30th April, 1959. Nearly all these carcases were examined bacteriologically and histological sections of various tissues taken. In each disease, the number of cases examined post-mortem and the number of farms from which they came is mentioned so that some idea of the severity of the outbreaks can be gathered, although it must be appreciated that all the deaths in each outbreak were not examined. Parasitic disease is unimportant in mink, since the animals are usually kept in wire mesh cages raised off the ground. McTaggart (1959) found in a series of over 200 mink (many from the series considered in this paper) that 89 per cent. were parasite-free. Of the 11 per cent. with subclinical infestation, 4 per cent. showed evidence of coccidiosis, while *Cryptocotyle lingua* (McTaggart, 1958) accounted for the bulk of the remaining cases.

No attempt has been made to survey the extensive European and North American literature on mink disease; readers are referred to Kennedy (1951) and the annual *Fur Farm Guide Book of the American Fur Breeder* for adequate summaries of the literature.

TABLE I

FUR BREEDERS' ASSOCIATION OF THE UNITED KINGDOM CENSUS RETURNS
(Number of mink on members' farms on 31st January of each year)

Year	1953	1954	1955	1956	1957	1958	1959
Farms with 1– 10 females ..	17	39	24	30	25	29	30
,, ,, 11– 25 ,, ..	31	44	54	33	46	61	77
,, ,, 26– 50 ,, ..	18	33	32	55	49	73	94
,, ,, 51–100 ,, ..	14	12	23	38	52	54	80
,, ,, 101–150 ,, ..	8	9	11	14	26	32	38
,, ,, 151–300 ,, ..	10	9	17	20	18	16	42
,, ,, 301–500 ,, ..	—	—	—	8	7	6	10
,, ,, 501–1,000 ,, ..	—	—	—	—	5	7	5
,, ,, over 1,000 ,, ..	—	—	—	—	—	—	2
Total Number of Farms	98	146	161	198	228	278	378
Number of Mink							
Standard	4,488	6,124	8,782	13,406	13,769	12,778	18,185
Silverblu ..		1,410	2,246	3,119	4,537	5,448	6,243
Sapphire ..	1,735	269	495	1,353	2,720	4,041	5,513
Pastel		597	773	1,877	3,472	4,510	7,854
Other Mutations		269	465	1,076	1,699	2,812	6,764
Total Number of Mink	6,223	8,669	12,761	20,831	26,197	29,589	44,559

TABLE II
ANNUAL AND COLOUR PHASE DISTRIBUTION OF MINK DISEASES

	1949	1950	1951	1952	1953	1954	1955	1956	1957	1958	1959*	Total 1949–59	Standard	Silverblu	Sapphire	Pastel	Others	Unknown
Nutritional Deficiency	1	4	8	4	9	7	36	49	34	16	—	168	110	27	5	11	—	15
Non-specific Enteritis	1	2	—	—	—	4	4	14	5	4	—	34	9	9	1	6	2	7
Salmonellosis	—	—	—	—	—	12	2	5	1	—	—	20	8	4	1	2	3	2
Distemper	—	—	—	—	—	—	—	70	6	—	—	76	62	6	3	5	—	—
Chronic Interstitial Nephritis	—	1	1	—	6	3	5	10	16	5	—	47	39	3	—	1	—	4
Encephalomyelitis	—	—	—	—	—	—	2	3	4	2	4	15	8	1	1	1	1	3
Rhinitis	—	—	—	—	—	—	2	4	3	3	—	12	5	1	2	—	—	4
Foreign body in Alimentary Tract	—	—	—	—	2	6	10	11	13	8	1	51	34	8	1	2	—	6
Pneumonia	—	—	2	—	—	4	3	1	1	—	—	11	5	4	—	1	—	1
Chilling	—	—	—	—	—	—	19	6	11	2	—	38	33	4	—	—	—	1
Urinary Calculi	—	—	—	—	4	4	7	5	10	5	2	37	20	9	2	1	1	4
Metritis	—	—	—	—	2	3	5	1	7	6	—	24	14	6	1	2	—	1
Food Poisoning	—	—	—	25	—	—	8	—	—	2	1	36	28	7	—	1	—	—
Plasma-cell Leucosis†	—	1	—	—	2	—	1	1	19	13	—	37	15	6	5	5	4	2
Pleurisy†	2	1	—	—	—	—	1	2	2	3	—	11	3	1	3	1	1	2
Tuberculosis†	—	—	—	—	—	6	4	3	7	8	4	32	6	10	8	3	1	4
Aleutian Disease†	—	—	—	—	—	—	—	—	4	5	4	13	1	1	7	1	2	1
Fusiformis Infection†	—	—	—	—	1	—	—	—	20	3	—	24	5	2	16	—	—	1
Other Diseases	3	9	12	3	16	15	34	20	51	33	14	210	92	18	7	10	9	74
Total Mink Autopsied	7	18	23	32	42	64	143	205	214	118	30	896	497	127	63	53	24	132

* January to April.

† Diseases apparently influenced by colour phase.

Nutritional Deficiency Diseases

The literature concerning experimental nutritional requirements in mink is well summarised in "Nutritional Requirements for Foxes and Mink" (*Nat. Acad. Sci.*, N.R.C. No. 7, 1954). It is pointed out in this publication that diets having a high content of unsaturated fatty acids and a low content of vitamin E give rise to Yellow Fat Disease—synonyms, non-suppurative paniculitis, Watery Hide Disease, steatitis and fatty degeneration of the liver. Thiamine deficiency causes emaciation, black diarrhoea, fatty degeneration of the liver and weakness, with eventual paralysis and death. Folic acid deficiency results in fatty degeneration of the liver and haemorrhagic gastro-enteritis. The basic components of the diet of mink in the United Kingdom are such that when fed in the wrong proportions a complicated pattern of nutritional deficiencies results, so that little is gained in attempting to separate which essential factor is most deficient.

The history in such outbreaks was that the animals had shown progressive loss of condition for up to two months. The fur had a moth-eaten appearance, and a green and slimy diarrhoea was commonly seen, becoming black before death. The males often showed "wet belly", that is, soiling of the fur by dribbling urine. Some animals exhibited a weaving motion, others were weak on their hind legs, and occasionally some died in fits. If the deficiency occurred during the mating period, many females were barren. The kits born on such farms did fairly well until the time of weaning at the end of June or the beginning of July, but once they depended exclusively on solid food for nutriment, deaths began to occur. The peak death-rate in the kits was reached in October, that is, at the time of "furring up". In adults the peak incidence of deaths occurred in females in February, related to the common practice of partially starving them to ensure that they were not over-fat prior to mating. When successfully mated, the females were often given supplementary rations, whereas the males were not necessarily fed such supplements, and if the diet was deficient then losses were observed in the male after the mating period at the end of March. A second peak death-rate in the females occurred shortly after weaning, especially in animals with large litters, that is, seven or more kits. This is often spoken of as "death from nursing sickness". I have never encountered nursing sickness deaths except where there was evidence of nutritional deficiency.

Post-mortem Findings (Figs. 1 and 2)

148 autopsies from 53 farms.

If the degree of imbalance was slight, or the feeding period of the abnormal diet was short, then some yellowish fat was present in the fat depots under the skin and in the abdominal cavity. If the imbalance was more prolonged or more severe, it resulted in an emaciated carcase and such carcases sometimes showed marked subcutaneous oedema which was often haemorrhagic.

On histological examination of the spleen there were numerous megakaryocytes, indicating extramedullary haemopoiesis (nutritional anaemia) and in the brain small peri-arteriolar haemorrhages were sometimes seen.

The diagnosis of nutritional deficiency was confirmed by noting the response of the animals to an improved diet. Most of the cases of nutritional deficiency encountered were due to feeding excessive amounts of fish and almost no liver. If about 50 per cent. of the ration was composed of fish offal, fresh liver at 5 per cent. of the diet was adequate but 10 per cent. was desirable; fish offal at 80 per cent., however, could not be compensated for by 15 per cent. fresh liver. It should be noted that fish offal is not always of constant nutritive value and that certain samples are composed mainly of fish heads and back-bones with very little meat attached to them.

FIG. 1.—Nutritional Deficiency. Note the emaciation, pale (fatty) liver and kidneys and haemorrhage into the intestine.

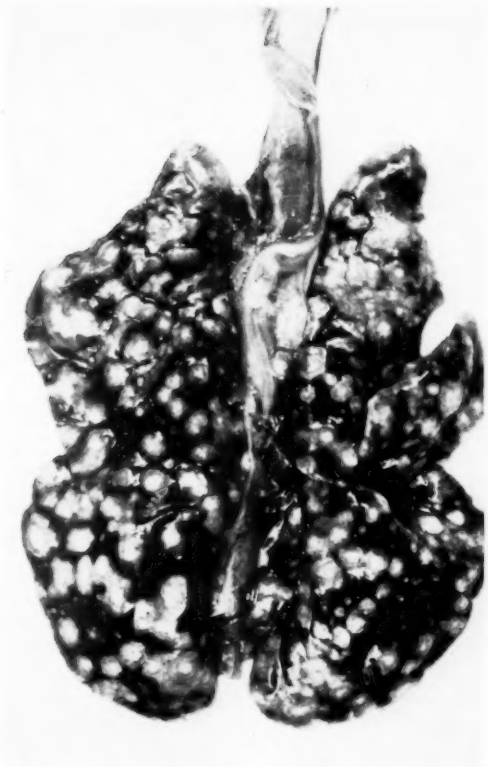

FIG. 2.—Nutritional Deficiency. *Lung*. Note the pale subpleural foci, especially in the dorsal diaphragmatic lobes of the lung, composed of macrophages having a foamy cytoplasm (cf. Fig. 6).

FIG. 3.—Distemper. Thickened, crusty, ulcerated lesions on the pads of all four feet. Such lesions do not occur in every case of distemper.

FIG. 4.—Distemper. Note the crusty lesions round the muzzle, purulent discharge round the eyes and "puffed-up" face.

FIG. 5.—Foreign Bodies in the Mouth. Note portion of wood wedged across the hard palate—a common site for this to occur because of the position of the last two molars. Also note a marker in a puncture wound behind the last upper cheek tooth, similarly the most common site for this lesion.

FIG. 6.—Tuberculosis. *Lung*. Yellow caseous foci in all lobes, having a congested border surrounded by a translucent zone (cf. Fig. 2).

(*a*) *Diet Too Coarse*

20 autopsies from 6 farms.

The mink has a small stomach and a simple intestine. It is obvious, therefore, that the diet must be easily digestible. A similar disease picture to that produced by nutritional deficiency was found on farms where the diet was either too fibrous (for example, bruised oats) or too coarse (for example, tripe diced by hand, not minced). A diagnosis can be made from the fact that the contents in the rectum closely resemble those in the stomach. Sometimes the condition occurred in the autumn associated with the maturing kits eating a full ration. The total food consumption on the farm therefore rose, and as high protein food was not obtainable the farmer increased the bulk of the ration by feeding cereal up to one-third of the total. With normal mink cereals the trouble was corrected by cooking prior to feeding.

(*b*) *Starvation* (accentuating a borderline dietetic deficiency)

9 autopsies from 3 farms.

On one farm, the mink were fed only 3 oz. each to "thin them down for mating". Deaths ceased when the food was increased to 8 oz. Another farm experienced losses as a result of starving the mink one day each week.

I have noted on several occasions that any disease which resulted in the mink not feeding very well for a week or more produced a picture very similar to that described in nutritional deficiency. The other lesions present in such carcases and the general history of the outbreak enabled a differential diagnosis to be made. In the following diseases where such changes occur, an asterisk has been inserted after the heading.

Enteritis

32 autopsies from 15 farms.

There were slightly more cases in the summer than at other times of the year. The animals showed a green or grey mucoid diarrhoea for two weeks or more. There was loss of condition and the coat became staring. Most of the animals recovered (the mortality rate was less than 1 per cent.) but a proportion relapsed ten days to a fortnight following an apparent cure. Throughout the farms irregular groups of animals were affected in waves, but not necessarily in neighbouring pens or rows.

On post-mortem examination the animals were seldom emaciated. The intestinal mucosa was somewhat thickened, there was excessive mucin production and the mesenteric lymph nodes were hyperplastic.

We have been unable to determine the cause of the non-specific enteritis. Paracolon organisms were not thought to be of importance, because in three outbreaks from which eleven carcases and fifty faeces samples were examined, paracolon organisms could be recovered from only a third of these samples (*Salmonella dublin* once and *Salmonella cholerae suis* once).

In none of these cases could helminths, protozoa or nutritional imbalance be incriminated. Histological examination of the pancreas showed no abnormality, and in one case examined, the faecal fat was 83 per cent. split. There was no evidence histologically (all cases) or haematologically (one case) to suggest that this enteritis was related to the virus enteritis described by Schofield (1949).

Salmonellosis

20 autopsies from 8 farms (*S. dublin* and *S. typhimurium* each from 4 farms).

This disease usually produced sudden deaths in kits, although occasionally they were seen ill for a few hours before death. For example, on one farm, liver was in short supply and so poultry waste was used as a supplement to the diet. Four days later a few kits died. Within sixteen days 135 kits out of 1,500 on the farm had died, the kits being forty-five to fifty-four days old. Pens throughout the farm were affected, involving most families, but only the weaker kits in each litter died. No adults showed any illness. Although *S. dublin* was recovered from seven out of ten mink examined, autopsy revealed very little of significance. Sulphamezathine treatment resulted in the control of the outbreak.

In adults a green diarrhoea was usually recorded and here the disease appeared to be less fatal. When salmonellosis resulted in the death of adults following prolonged diarrhoea, the diarrhoea usually changed from green to the black faeces of nutritional deficiency.

It is probable that salmonellosis only becomes a problem at times of stress, that is, at mating, kittening and weaning of the kits.

Distemper

76 autopsies from 2 farms (see Figs. 3 and 4).

The history of the first farm illustrated the important features of the disease and will therefore be described. On 18th October an adult Pastel male exhibited one very sore foot; by the 21st, all four feet were thickened and sore and the animal was destroyed. On that day, another Pastel male kit showed a "puffed-up" face and was off its food. The pads of the feet were thickened and sore and somewhat cracked. This animal died on 22nd October. On the same day a third Pastel male kit was off its feed, had feet lesions, scabs around its muzzle and a purulent discharge from the eyes. This animal died on 27th October. Between 27th October and 12th December, 68 animals (56 kits and 12 adults) died out of a total stock of 1,450 mink. At the beginning of the outbreak the majority of the animals were found dead. Towards the end of November, however, some of the animals developed fits and were off feed for several days prior to their death or destruction.

In the early stages of the outbreak, very few abnormalities were detected on post-mortem examination. In the later stages, however, pneumonia was present and one case of pleurisy was found. The animals which had been off their food for over a week showed a picture similar to that described in nutritional deficiency.

Intracytoplasmic inclusion bodies in the epithelium of the bronchi of the lungs, bile ducts, renal pelvis and urinary bladder were inconstant.

Bacteriological examination of forty of these carcases showed the presence of *S. dublin* in only four cases.

All the mink were vaccinated with a sub-standard egg-adapted distemper virus on 25th, 26th and 27th

October. Thirty affected animals were re-vaccinated on 30th October and twenty-five animals were re-vaccinated for the third time on 9th November. As a result of this, it would appear that about twenty animals which were affected clinically recovered. It is difficult to assess how severe this outbreak would have been had it not occurred at pelting time, since the owner pelted many animals which he thought were slightly off colour.

Chronic Interstitial Nephritis

47 autopsies from 11 farms.

The usual history of animals dying from this condition was that they had been off food for some considerable time, had an increased thirst and had progressively lost condition. Occasionally the mink died in convulsions, or exhibited posterior paralysis for a few days prior to death.

On *post-mortem* examination, the picture was similar to that described in the emaciated nutritional deficiency disease case, except that the kidneys were pale and the surface pitted. On cut surface, there was an irregular pale streaking with occasional small haemorrhages. Sometimes the kidneys were smaller than normal.

No constant bacteria could be recovered from the carcases on cultural examination and serological examination failed to reveal agglutinins against *Leptospira ictero haemorrhagiae* and *Leptospira canicola* in two cases. Three of the cases occurred on the farm with the large distemper outbreak two years prior to that outbreak (one of these was examined serologically for distemper with negative results). Nineteen of the cases occurred on the same farm from two to twelve months after the distemper outbreak (one of these cases was positive serologically for distemper, but two others were negative). This would suggest that distemper was not the only cause for this condition.

Kennedy (1951) mentions a similar kidney lesion associated with nutritional imbalance and, in fact, some of our cases occurred in animals which had survived a nutritional deficiency disease.

Plasma Cell Leucosis

37 autopsies from 19 farms (cf. Table II—mutation colour phases).

The history of animals dying from this complex was similar to that noted under chronic interstitial nephritis. The post-mortem picture was rather similar except that the liver, instead of being yellow and fatty, was enlarged, orange and mottled and somewhat firmer than normal. The spleen was often enlarged and occasionally ruptured during the pelting process.

Bacteriological examination was usually negative for pathogens, *Erysipelothrix rhusiopathiae* being isolated in one case and coliform organisms, streptococci and staphylococci occasionally. Serological examination for the presence of distemper was negative in the one case examined.

One case took place two years before and one two years after the major distemper outbreaks described above. These findings would suggest that there is no obvious relationship between distemper and this complex of diseases.

Aleutian Disease

13 autopsies from 10 farms (cf. Table II—Sapphire and related colour phases).

The history, *post-mortem* and histological findings in these cases were very similar to those described under plasma-cell leucosis, except that the animals often showed some degree of jaundice.

Again, bacteriological examination was usually negative for pathogens, but a *Pasteurella* spp. was recovered in one case and *Salmonella cholerae suis* in another instance. None of these cases occurred on farms on which distemper was known to exist or to have existed.

The work of Helmboldt and Jungherr (1958) would suggest that the above classification of chronic interstitial nephritis, plasma-cell leucosis and Aleutian disease was merely the result of setting names to various stages of one condition (Table III). Until more research has been carried out on this problem,

TABLE III

SUMMARY OF THE HISTOLOGICAL FINDINGS
in Distemper, Chronic Interstitial Nephritis, Plasma-Cell Leucosis, Aleutian Disease and Encephalomyelitis

		Distemper	Chronic Interstitial Nephritis	Plasma-cell Leucosis	Aleutian Disease	Encephalomyelitis
Periarteriolar plasma-cell cuffing and hyalinisation, especially in lung, heart and stomach		*	*	§	‖	*
Brain	Focal demylination and gliosis	†	*	*	*	‖
	Perivascular round-cell cuffing	†	†	†	†	‖
	Plasma-cell foci in meninges	*	†	‡	‡	†
Liver	Periportal fibrosis with plasma cells	*	*	§	§	†
	Hyperplasia of bile ducts	*	*	*	§	*
Kidney	Hyaline degeneration	*	§	§	§	*
	Interstitial and periglomerular fibrosis	*	§	§	§	*
	Interstitial plasma-cell foci	†	§	§	§	*
	Casts in Bowman's capsule and cortical tubules	*	§	§	§	*
Spleen	Hyaline degeneration	*	†	‡	‡	*
	Plasma cells in red pulp	*	§	‖	‖	†

* Not seen.
† Sometimes seen.
‡ Slight.
§ Moderate.
‖ Marked.

however, it is difficult to decide whether these are various stages in one disease, or different reactions of the host to different agents, or whether they are reactions of different hosts to the same agent.

Encephalomyelitis

15 autopsies from 13 farms.

The usual history in these cases was that the animal died either in a screaming fit or after a week of progressive paralysis.

On *post-mortem* examination, the carcase was well developed and showed no obvious abnormality.

Three of the cases in this series occurred on farms in association with an outbreak of distemper (one case four months after, one case nine months after and one case a month before the outbreak). The remainder occurred as isolated cases on farms on which no evidence of distemper existed.

The preceding four diseases could all be the result of virus infections (see Table III). Until more is known about the response of the mink to distemper and other virus infections, there seems little point in speculating further on this matter.

Rhinitis

12 autopsies from 9 farms.

The usual history in these cases was of the mink being found dead, sometimes after having shown a nasal discharge for a day or two.

On *post-mortem* examination the nasal pathways contained considerable amounts of red or yellow muco-purulent material. The carcase was well nourished, but the liver and kidney showed varying degrees of fatty change and the spleen was often slightly enlarged. In five of the cases a meningitis was present, especially on the olfactory lobes of the cerebrum.

There was usually evidence of a terminal septicaemia, the commonest organism recovered being a coliform, although streptococci and staphylococci were also isolated and on one occasion *E. rhusiopathiae* was cultured.

Foreign Bodies in the Alimentary Tract

It is well known that mink frequently get fish-bones or portions of wood from their nest boxes wedged in the mouth. Most mink farmers recognise this condition by the animal pawing at its mouth and remove the bones themselves. Sometimes, however, such animals were not attended to promptly and subsequently died.

(*a*) *Foreign Body wedged between the two Upper Carnassial Teeth* (Fig. 5)

10 autopsies from 10 farms.

The usual history was that these animals had been off food for one week, had diarrhoea and weakness of the hind legs.

On *post-mortem* examination, necrosis of varying extent was associated with a foreign body. In one of our cases where *Fusiformis necrophorus* was present, the necrotising lesion had penetrated into the nasal cavity and so into the brain.

(*b*) *Puncture Wounds in the Mouth Region* (Fig. 5)

24 autopsies from 20 farms.

These wounds represented the site of penetration of a foreign body although the foreign body was not present at autopsy.

The usual history was that these animals had been ill for from two weeks to three months with recurrent abscesses bursting below the eye.

In sixteen cases there was a rhinitis, in eight of which the infection had spread to cause a meningitis, and in five cases had spread to cause a pneumonia.

The organisms commonly recovered were streptococci and staphylococci, but in one case *E. rhusiopathiae* was isolated.

(*c*) *Paradontal Disease*

6 autopsies from 6 farms.

These cases are included in this section because they had a history very similar to the cases of fish-bone wedged in the mouth. In adults, the disease was usually connected with damage to the canine teeth, while in kits it occurred in association with the eruption of the permanent teeth at approximately two months old.

In all cases a rhinitis was present, and in three of the mink the infection had spread to cause a meningitis and once also to cause a pneumonia.

(*d*) *Intestinal Obstruction*

5 autopsies from 4 farms.

The history presented with these animals was that illness had been noted for up to one month.

The obstruction was caused by fur-ball or wood shavings from the bedding and in three cases occurred in the duodenum, while the remaining two occurred 3 cm. from the anus.

(*e*) *Perforation of the Intestine*

6 autopsies from 4 farms.

These animals were presented with a history of illness of a few days' duration and the abdomen becoming increasingly pendulous.

At autopsy a fish-bone perforation was observed in the duodenum with an associated peritonitis.

Primary Pleurisy

11 autopsies from 9 farms (cf. Table II—mutation colour phases).

The general picture was one of nutritional deficiency associated with the fact that these animals were usually ill for a week or more prior to death.

The pleurisy tended to be unilateral and fibrinous.

The organisms recovered were variable, being mainly coliform organisms, occasionally *Pasteurella* spp. and in one case *Corynebacterium pyogenes*.

In the adults, infection was usually associated with the refusal of the animal to allow mating and the infliction of a penetrating wound in the ensuing struggle. The condition also occurred in individuals in unseparated litters following bite wounds by large kits with well-developed canine teeth.

Pneumonia

11 autopsies from 6 farms.

The history with these animals was that they had been off food for some time with progressive respiratory distress, and at autopsy, in addition to the nutritional deficiency picture, there were irregular and extensive areas of red-grey consolidation in the

lungs with variably sized white purulent foci scattered throughout.

The organisms recovered were either haemolytic staphylococci or streptococci.

Chilling

35 autopsies from 13 farms.

The usual history in such cases was that the animals died the day after getting wet through. Five of the cases occurred in January, associated with melting snow wetting the bedding in the nest boxes. These animals had been ill for some five days prior to death. The remaining deaths took place during the summer months associated with heavy rain-storms either soaking the straw in the nest boxes while the mink were still on solid floors, or soaking groups of weaned but not separated mink kits. Such deaths did not occur after the kits had been separated into individual boxes.

The animals were well nourished, and on post-mortem examination showed little change apart from marked congestion, oedema and haemorrhage in the lungs with an early broncho-pneumonia.

The majority of cases revealed no pathogens on cultural examination although on occasion, coliform organisms, staphylococci and streptococci were recovered.

Heat Stroke

5 autopsies from 5 farms.

This occurred in very hot weather in June and July. Only the kits were involved, being found dead in the straw of the nest box, while those in the run were not affected.

On post-mortem examination the findings were intense congestion, oedema and haemorrhage from the lungs.

Urinary Calculi

37 autopsies from 25 farms.

Only four of these were found in females. Deaths from this cause in kits started in June, reached a peak in July or August and continued until pelting in December. In adults, cases were found in all months of the year, but the highest incidence was in March.

The usual history was of the animal being noted off food for at least one day and sometimes there was a loss of condition with death in fits or after paralysis. The fur around the hindquarters was matted due to dribbling of urine. The general picture was one of nutritional deficiency, but, in addition, the bladder was distended with cloudy red urine and the mucosa often resembled red velvet.

In eleven cases the calculi were of the size of sand grains and, in these, bacteriological examination failed to reveal the presence of pathogens. In twenty-one cases the calculi ranged from 0·25 to 2 cm. diameter and were multiple. The kidneys often showed some degree of hydronephrosis (haemolytic staphylococci were recovered from all tissues in most of this group). In three animals, calculi were present in the renal pelvis as well as in the bladder.

Of fifteen calculi examined, thirteen had a chemical composition of ammonium magnesium phosphate; the other two were a combination of ammonium magnesium phosphate and calcium and magnesium hydrogen phosphate.

Metritis

24 autopsies from 16 farms.

All these cases occurred between the middle of April and the end of May. The carcases were well nourished and showed, in addition to the uterine changes, fatty change in the liver and kidney and enlargement of the spleen due to extramedullary haemopoiesis.

In twelve cases, coliform organisms were isolated from the uterus and in five of these there was a coliform septicaemia. Beta haemolytic streptococci were recovered in three cases, *Pasteurella* spp. twice and *S. dublin* once.

In five of the mink there had been an abortion. There were no kits in the uterus, but a considerable amount of green or brown pus was present. These animals had been ill for two weeks or more prior to death.

Six of the cases were found dead with the kits in the uterus undergoing varying degrees of decomposition.

In five females, which died three days after the birth of some kits, further foetuses were retained in the uterus and in one case foetuses were found in the abdominal cavity due to rupture of the uterus.

Three animals died five days after the birth of normal kits and showed a peritonitis associated with rupture of the uterus.

Five cases showed marked haemorrhage and early necrosis between the foetal and maternal placenta, the foetuses still being present in the uterus but undergoing degenerative change. These animals died after one week of illness.

Tuberculosis

32 autopsies from 22 farms (cf. Table II—mutation colour phases).

All cases had a history of being very thin for some time, but were obviously ill for only two to three weeks before death. Some animals sneezed and coughed for up to a month prior to death and some had a history of recurrent abscesses in the head and shoulder regions (involvement of the lymph nodes).

The general picture on post-mortem examination was one of infection via the intestinal wall, with widespread lympho-haematogenous dissemination from an incomplete primary complex in the mesenteric lymph node (Fig. 6). Although the liver and spleen were enlarged, discrete foci were difficult to recognise macroscopically; histological examination, however, showed extensive involvement.

In the majority of cases very numerous acid-fast organisms were present in these lesions. Cultural and biological examination of material from fifteen of these cases showed the organisms were of the bovine strain, suggestive of origin from infected meat in the diet.

Two mink from one farm (not included in the above cases) showed a similar post-mortem and histological picture, but no acid-fast organisms or other causative agents could be demonstrated.

Mucor Mycosis

4 autopsies from 3 farms.

In two cases lesions of similar macroscopic character to those described in tuberculosis were

present, but on histological examination numerous fungal hyphae were detected. The other two cases showed a purulent rhinitis and a meningitis associated with fungal hyphae.

The diagnosis was made from the character of the hyphae—the fungus was not isolated.

Fusiformis necrophorus Infection

24 autopsies from 18 farms (cf. Table II—mutation colour phase).

The usual history was that the animal was off food and dull for up to a week before death.

On post-mortem examination, the carcase was well nourished but the fat was jaundiced in appearance. The liver was pale and fatty with irregular and friable areas of necrosis, yellowish-pink in colour and covered by a marked fibrin deposit. While these necrotic areas can occur in any lobe they were most common in the right lateral lobe. The spleen was considerably enlarged. In a proportion of cases the infection had penetrated through the diaphragm to involve the diaphragmatic lobes of the lung and in the majority of cases there had been spread to the brain.

Although the stomach and duodenum were congested and haemorrhagic and the mesenteric lymph nodes enlarged and congested, no Fusiformis organisms could be demonstrated in these sites. In the mink there were very numerous Fusiformis organisms in these liver lesions and almost no attempt to form a reaction zone, in contrast to the common picture in the bovine animal.

Suspected Food Poisoning

36 autopsies from 5 farms.

In each of these outbreaks the diagnosis was made on circumstantial evidence—not by the demonstration of the responsible toxin or organism.

These outbreaks occurred when the weather was extremely hot and the history was that the feed had been left lying some hours before being distributed. Within twelve hours of giving the suspected feed, the mink started to vomit green material streaked with blood, many developed posterior paralysis and a variable proportion died in convulsions. On one farm 125 out of 400 animals were paralysed and twenty-five of these died. On another farm only twelve animals out of 110 became ill and died.

On post-mortem examination, the animals appeared well nourished, all viscera were somewhat congested and the alimentary tract showed varying degrees of intense congestion and haemorrhage.

Chemical Poisoning

(*a*) *Red Squill*

2 autopsies from 1 farm.

Two animals were found dead shortly after Red Squill had been used as a rat poison on the farm.

(*b*) *Strychnine*

2 autopsies from 2 farms.

The history provided was of sudden death, with the other animals on the farm showing posterior paralysis and being uninterested in food. This occurred after the incorporation of a strychnine tonic in the feed.

(*c*) *Suspected Carbon Tetrachloride Poisoning*

4 cases from 1 farm.

On this farm, day-old chicks formed the whole of the diet for one meal and, out of 110 animals, on the next day nine were very ill with thirst and vomiting. Subsequent history indicated that the day-old chicks (which normally formed 20 per cent. of the ration) were usually drowned, but on this occasion some of the chicks had been destroyed by carbon tetrachloride.

(*d*) *Hexoestrol Poisoning*

9 cases from 2 farms.

On the first farm sixteen mink kits were implanted with 15-mgm. tablets of hexoestrol on 7th October. By the beginning of November several animals were ill and showed "wet belly". By 21st November all the sixteen mink kits were dead or had been destroyed. On the second farm, 5-mgm. hexoestrol implants were used, starting on 17th September. By 5th October animals began to develop "wet belly" and loss of flesh, culminating in death on 19th October.

The animals submitted to autopsy at the beginning of the outbreaks were well nourished, but those at the end of the period were very thin. The liver and kidneys showed fatty change, but the most dramatic feature was an acute haemorrhagic inflammation of the urinary tract from renal pelvis to bladder.

Miscellaneous

The remaining types of disease occurred in small numbers and are summarised as follows:

Bite Wounds—6 autopsies from 6 farms (usually in unseparated kits or at mating).

Abscesses—6 autopsies from 4 farms (4 in the para-anal sacs).

Malformations—6 autopsies from 5 farms (hydrocephalus; truncus communis in heart; inguinal hernia; agenesia of right hind foot; obesity).

Tumours—5 autopsies from 4 farms (teratoma—ovary; teratoma—mediastinum; lymphosarcoma; hepatocellular carcinoma; visceral cavernous haemangioma).

Metastatic Calcification of the Kidney—5 autopsies from 3 farms.

Handling Trauma—4 autopsies from 3 farms.

Atheroma Aorta with Rupture—1 autopsy.

Staphylococcal Bacterial Valvular Endocarditis—1 autopsy.

Staphylococcal Suppurative Mastitis—1 autopsy.

Acute Cystic Cholangitis—1 autopsy.

Erysipelothrix rhusiopathiae Septicaemia—1 autopsy.

Ruptured Gall-bladder—1 autopsy.

Coccidiosis—1 autopsy.

Hair Loss—3 autopsies from 2 farms.

Sterility—2 autopsies from 1 farm.

Starvation in 1-month-old Kits—26 autopsies from 7 farms.

Probable Nutritional Deficiency but no details of Diet—40 autopsies from 33 farms.

Emaciated Carcases (cause of death unknown)—29 autopsies from 16 farms.

Unknown: No abnormality detected at post-mortem—18 autopsies from 15 farms.

Post-mortem change very advanced—8 autopsies from 8 farms.

Normal Mink from a Farm on which a Deficient Diet had been Corrected—8 autopsies.

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References

HELMBOLDT, C. F., and JUNGHERR, E. L. (1958). *Amer. J. Vet. Res.*, **19**, 212-22.

KENNEDY, A. H. (1951). "The Mink in Health and Disease", *Fur Trade Journal of Canada*, Toronto, Ontario.

MCTAGGART, H. S. (1958). *Nature*, **181**, 651.

——. (1959). In Press. J. Parasit. and J. Helminth.

SCHOFIELD, F. W. (1949). *No. Amer. Vet.*, **30**, 651-4.

Diseases of Chinchillas

BY

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UNLIKE most of the animals with which veterinary surgeons have to deal, the chinchilla is one of very recent domestication, and many members of the profession are unfamiliar with this animal and its diseases. This is not surprising, as domestication in America dates back for only thirty years or so and it must be less than that in the United Kingdom. In fact, it is only in very recent years that the chinchilla has been popularised, to any extent, in our daily Press and periodicals.

The appeal seems to be economic rather than sentimental, as the comparatively high cost of chinchillas would seem to preclude their being kept as pets primarily. The much-vaunted simplicity of breeding, feeding and making money out of chinchillas has, I would say, its greatest appeal to semi-retired people and to those wishing to make a "bit extra" in their spare time.

Coupled with the potential profit is the fact that small outhouses or gardens may be used for chinchilla husbandry and little is required in the way of staff and equipment. It is even said that chinchillas can be kept in basements and box-rooms.

Whether domestication and concentration in unnatural surroundings have increased these animals' susceptibility to disease or rendered them liable to diseases to which they were not normally susceptible is a matter for conjecture; but I feel that one should bear in mind that they originated from the Andes mountains—some 10,000 ft. above sea-level. Those which live at lower levels are said to have poorer coats.

Whilst most animals can be adapted to change of climate, the optimism with which this is carried out sometimes is most surprising. I know of one case where chinchillas were sent from California by air via the North Pole to Scandinavia, then by air to London and by train to North-east England in winter. A number did not survive.

Although management is outside the scope of this paper, a brief reference to chinchillas in general might assist those veterinary surgeons unfamiliar with the animal.

The chinchilla is a rodent. The markings of both sexes are the same and the female is slightly larger than the male. There are two species, *C. laniger* and *C. Brevicaudata*, the latter being slightly larger and darker.

For the size of the animal the gestation period seems unusually long—about 112 days in the case of *C. laniger* and as much as 128 days in the larger species. The male is left with the female during parturition and he may serve her soon after that event. In domestication the average litter consists of two, whereas in the wild state it is said to be four. The young are weaned at about three months of age.

Diseases

I would have preferred that this paper had been entitled *some* diseases of chinchillas. It is almost as impossible to cover the ailments of chinchillas in one paper, as it would be for any other animal.

My experience has been confined mainly to naked-eye post-mortem examinations with some laboratory investigation, chiefly of a bacteriological nature. I took to this work with some reluctance, but appeals for help came in from so many veterinary surgeons that it became obvious that something—however little—had to be done to try to get to the bottom of some chinchilla ailments.

It seems to me that the symptomatology of the chinchilla has either not been studied, or it is a fact that they are very prone to die suddenly without symptoms. To some extent this postulation is borne out by my figures. In forty-seven case histories accompanying carcases for post-mortem examination, no fewer than thirty-three had a history of sudden death, or, at the most, an indefinite illness of a few hours' duration. A frequent history was that the animals were vigorous and feeding well, but were found dead in the morning. In many of these cases, veterinary surgeons were not called in until after one or several deaths had occurred. No doubt professional observation would have elicited a few symptoms, but in my experience these have been lacking.

Materials and Methods

These were quite simple, and consisted of naked-eye post-mortem examination of all organs including the brain. As a routine measure, swabs were taken from heart blood, liver, lung and bowel contents. Cultures were made in nutrient broth, horse blood agar, MacConkey's medium and desoxycholate citrate agar. Kligler iron agar and sugar fermentation tests were used in the identification of some of the bowel bacteria. Cultures were made from other organs as necessary. In most cases the bowel contents were examined microscopically for helminth ova.

General Lesions

In 1,000 post-mortem examinations carried out prior to 1949, Brenon (1953) in the U.S.A. found 25 per cent. had lobar pneumonia, 23 per cent. had epidemic gastro-enteritis and 12 per cent. had acute impaction. Between 1949 and 1952 a further 1,000 post-mortems were performed with 22 per cent. pneumonias, 24 per cent. enteritis, but impactions had risen to 20 per cent. (Brenon, 1955). In these statistics Brenon did not publish bacteriological findings.

In only forty-seven post-mortems, I found that the predominant lesions were (a) enteritis, mostly in the small intestine, eleven cases; (b) blood-tinged transudate in thoracic or abdominal cavities, nine cases; (c) lobar or broncho-pneumonia, seven cases; (d) impaction, five cases; (e) Pasteurella pseudo-tuberculosis, four cases; (f) intussusception, three cases; (g) peritonitis, two cases, and the remainder were fatty

degeneration of the liver, metritis, one case of meningitis and one of tuberculosis.

In a number of these cases death terminated in septicaemia.

In considering the various pathogens isolated from chinchillas and attempting to relate them to the histories of "sudden death" coupled with the post-mortem lesions, I have found it difficult to assign any particular importance to one group of septicaemic organisms over another. It is often a case of a "mixed bag".

When petechial haemorrhages are found throughout internal organs and pathogenic bacteria are isolated from heart blood, one is on pretty safe ground in making a diagnosis of septicaemia—a condition to which the chinchilla seems to be particularly susceptible, with very rapid death.

Unfortunately, in many cases the exact cause of death is far from clear, and I would say that this is most common in the group suffering from inflammation of the small intestine, with or without a serous or blood-stained transudate of the thoracic and abdominal cavities.

Many of these cases seem complicated by defective feeding, and/or the presence of *Giardia* spp. trophozoites in the duodenum and jejunum.

Streptococci and Staphylococci

Both haemolytic and non-haemolytic strains are isolated frequently from pneumonia, metritis, enteritis, peritonitis and septicaemic cases. Although there might be a modern tendency to decry or underestimate the pathogenicity of these organisms (as they can be isolated from healthy animals), I feel that in the presence of positive lesions they should be considered as pathogens. Perhaps they are opportunists.

Staphylococcal entero-colitis has been reported in the United States as a serious condition in chinchillas, and the authors (Wood *et al.* 1956) postulated that the feeding of commercial pellets containing 0·2 mg. of chlortetracycline per ounce may have influenced the balance of the normal bowel flora.

Pasteurella Pseudo-tuberculosis

This is a common infection of rodents, and I have encountered it in four cases from different chinchilla herds. Yellowish-white necrotic foci 1-3 mm. are usually found in the liver, but in one case the primary focus of infection was in the parotid salivary and lymph glands, and lesions were found in lungs, liver, spleen and kidneys. The typical Gram negative coccobacillus was isolated from all organs. On cursory examination, this disease can be mistaken for miliary tuberculosis, also for the abdominal form of listeriosis.

Leader and Baker (1954) reported two outbreaks of pseudo-tuberculosis in chinchillas and the disease was reproduced experimentally in a guinea pig and two chinchillas. It is interesting to note that the incubation period was about one week. The experimental animals died or were destroyed about a fortnight after receiving the cultures. They developed rough coats, lassitude, loss of weight and dyspnoea.

Thal (1953) in Sweden reported that an avirulent living strain of *Past. pseudo-tuberculosis* protected guinea pigs against challenge with virulent strains of the organism and this was confirmed by other workers. But when the Swedish strain was tried in an experiment on chinchillas, nineteen out of twenty animals succumbed to the typical disease within four weeks and the avirulent strain was found to be virulent for chinchillas. (National Chinchilla Breeders' Association, 1955.)

When this disease has been diagnosed, it is as well to check up on contamination of food and water supply by wild rodents.

Proteus spp.

I have made thirteen heart blood isolations from varying types of cases ranging from pneumonia to enteritis, metritis and intussusceptions. Obviously the connection is not clear. I consider there are pathogenic strains of *Proteus* particularly typical and atypical *P. morgani*, although *P. vulgaris* might be considered as a potential pathogen.

In several cases where *P. morgani* spp. had been isolated they proved to be highly pathogenic for mice. In one experiment, mice were killed within three hours following intra-peritoneal injection of a broth culture. This would seem to indicate that *Proteus* spp. may produce a powerful toxin, but further work is required on that subject. It is not clear whether a particular strain of *Proteus* spp. can acquire pathogenicity or whether it acts as an opportunist in tissues already damaged by other agents.

Pseudomonas aeruginosa

This is said to be a common pathogen of the chinchilla, and McIntyre (1952) states that on one ranch in the U.S.A. it was present in 100 per cent. of the fatal septicaemias of the new-born. It is also associated with abscesses, pneumonias, enteritis and septicaemias of adults. I have made only one isolation of this organism from heart blood, and in this case the immediate cause of death was an intussusception of the small intestine.

Listeriosis

The isolation of *Listeria monocytogenes* from domestic animals and laboratory rodents has been reported from various parts of the world, and it would seem to be fairly common in chinchillas in the U.S.A. In the encephalitic form there may be nervous symptoms accompanied by crying, salivation, twitching of groups of muscles particularly of the face and ears. Where the disease exists in the abdominal or septicaemic form the lesions may include petechial haemorrhages of internal organs, or, in more chronic cases, greyish-white foci in the liver and other organs closely resembling *Past. pseudo-tuberculosis*.

I have encountered one case with a history of being hypersensitive for two days before death (Stewart). There was acute congestion of thyroids, lungs, kidneys, liver and meninges. *L. monocytogenes* was isolated from liver and brain. The possibility of wild rodent carriers should be borne in mind.

Giardiosis

These protozoan flagellates are commonly found in the intestines of chinchillas. They are bi-nucleate and the body, which is pear-shaped, contains a sucker on

the ventral surface with which it attaches itself to the alimentary canal of the host. Excluding the flagellae, the body is about 15 microns in length. They are most commonly found in the small intestine but may invade the whole bowel and cysts may be found in the caecum and faeces. The pathogenicity of these protozoa is debatable.

In my opinion the finding of a serous or blood-tinged transudate in the abdominal (and sometimes in the thoracic) cavity is an indication of the possible presence of *Giardia* spp. In fifteen cases in which I found *Giardia* at post-mortem, nine had such transudates and the majority of these had an enteritis as well. I think it is interesting to record that in six of these cases with transudates, the heart blood and liver were found to be bacteriologically sterile. This does not prove the pathogenicity of *Giardia* spp., but in the absence of any other obvious case for the transudates, it might be considered as a possibility.

Intestinal Impaction

The caecum of the chinchilla seems rather large in proportion to the size of the animal and it is possible that bacterial digestion of rough foods might take place in that organ. Feeding of concentrated pellets with insufficient roughage might predispose to impaction.

The impaction may often be palpated through the abdominal wall. Lying on one side and stretching the legs are said to be symptoms suggestive of an impaction. The high incidence of impaction warrants careful inquiry into the feeding methods adopted in many herds. Research into chinchilla nutrition is needed.

Fur-chewing

This condition has been referred to as one of the most serious facing chinchilla husbandry in the U.S.A. (Gorham and Farrell, 1955). A fur-chewing animal represents almost a total loss, as it will not only chew itself but its mate and young as well. Sometimes the whole body will be depilated.

It has been attributed to (a) abnormal behaviour patterns associated with environmental stress—a sort of psychological maladjustment to domestication; (b) endocrine disturbances; (c) unpalatable foods; (d) too much protein; (e) vitamin deficiency; and (f) deficiency in sulphur amino acids, cystine and methionine.

A more natural form of husbandry providing greater freedom combined with fresh herbage might prove the answer to this problem and perhaps to many other problems facing the chinchilla breeder. Time will tell.

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References

BRENON, H. C. (1953). *J. A. V.M.A.*, **123,** 310.
——. (1955). *J. A. V.M.A.*, **126,** 222.
GORHAM, J. R., and FARRELL, K. (1955). *Proc. Amer. V.M.A.*, 228-34.
LEADER, R. W., and BAKER, G. A. (1954). *Cornell Vet.*, **XLIV,** No. 2.
MCINTYRE, R. W. Nat. Chinchilla Breeders' Association (1952). No. 19.
Nat. Chinchilla Breeders' Association (1955). No. 15. Report from State College of Washington.
STEWART, J. K. Personal communication.
THAL (1953). *Proc. XV Internat. Vet. Congr.* Part I. 67-70.
WOOD, J. S., *et al.* (1956). *Bull. John Hopkins Hosp.*, **98,** No. 6, 454-63.

THE SPEAKERS' INTRODUCTION

The Chairman, Mr. J. A. Dall, in introducing the authors, said that the two small animals to be discussed were very important nowadays, and there was a greater opportunity for co-operation between the owners, who were desperately anxious to have qualified advice, and the veterinary surgeons, if the veterinary surgeons in practice would use the basic knowledge which he had and apply it to these small animals. There was, of course, no question that it was essential for the practitioners to have very close liaison with the laboratories and *vice versa.* The speakers at the present session were primarily research workers. It was a most important duty of the practising veterinary surgeon to acquaint himself with the diseases as they were seen at *post-mortem* examination, since in these particular animals the most common diagnostic lesions were then seen. Frequently it was impossible to diagnose the disease condition from the symptoms exhibited when the animal was alive, so it was imperative to use laboratory facilities wherever possible.

Mr. Dall then asked Mr. Head to present his paper.

Mr. K. W. Head, in presenting his paper, showed some slides illustrating the paper, and concluded by adding that it was customary nowadays, when assessing an industry, to do so by mentioning its dollar-earning capacity. In 1958, 42,000 pelts had been exported to the U.S.A. for half a million dollars.

Mr. R. S. T. Bowden, in presenting his paper, said that in the introduction there was a reference to the natural habitat of the chinchilla and the climate from which it came, but he did not wish to rub in that aspect too much, because, as all of them knew, animals could be adapted fairly rapidly to climatic changes, but nevertheless it was a thing that should be borne in mind. However, he did feel that the chinchilla was being subjected to somewhat unnatural conditions. There was not much reference in the paper to management, since this was rather outside its scope, but he wished now to enlarge a little on that aspect.

A few days ago he had consulted a book on chinchilla diseases and ailments—he believed he was right in saying that it was the only book on this subject—and one or two points on matters of feeding and management had struck him as being rather peculiar. He therefore felt that might help if in the discussion practitioners who were dealing with these animals could give some enlightenment on these points.

For instance, it was quite obvious that one would not feed mildewed or mouldy hay or fermented foods through lack of cleaning the feed tins; but he felt that the chinchilla, being a herbivorous animal, should require plenty of fibre in its diet, and it seemed to him that this was not always the case.

To quote from the book to which he had referred, alfalfa, Timothy, orchard grass were considered to be a suitable diet; animal proteins should not be fed, in particular animal oils; this also included vegetables oils, nuts and nut cake. Pre-cooked foods, such as corn flakes, rolled oats, shredded wheat and dairy cereals should not be fed either; nor should lettuce, cabbage, kale, or clover.

He merely mentioned this to the meeting. He thought that it was a little odd. He felt that a great deal of work and clear thinking was required on the nutrition of the chinchilla.

Another aspect on which he had said very little in the paper was the general breeding of chinchillas. It struck one that the gestation period for such a small animal was unusually long, a factor which perhaps might come into the economics of this business. They were often paired together to get adjusted, and during the mating there was often quite a lot of fighting, with loose tufts of hair around the cage. Normally the female vagina was sealed tightly; at oestrum it opened, and there might be a secretion, and the nipples became reddened. After mating, a "stopper" or copulation plug was found—a sack-shaped membraneous structure

ejected by the female, which was probably the confirmed layer of the vaginal wall. Incidentally, it was suggested that where there was an aggressive female there should be a hide-out provided for the male. The oestrus cycle was said to be 24-28 days, and it seemed that May and September-October were the peak heat periods.

On symptomatalogy, as he had stated in the paper most of the cases that had come his way had been almost completely without history. Of 47 case histories, no fewer than 33 had a history of sudden death. That might require challenge to some extent, in that often the veterinary surgeon was called in after the death, and so one did not really get a clear picture; but it seemed to him that chinchilla was particularly liable to acute death.

He wished to enlarge a little on the streptococci and staphylococci, particularly with regard to staphylococcal entero-colitis. This had been reported by Wood in America, who had given a very good account of how some 50 out of 450 chinchillas had died over a period of a year or so when they were on a pellet feed containing 0.2 mg. of chlortetracycline per ounce. Wood had postulated that there was some affiliation between this and staphylococcal entero-colitis. Some recent work on this had come into his possession since he wrote the paper. Probaska, in the *Journal of Surgery and Gynaecology* (1959, 109, 1, p. 73) had written up this candition in man, and it was most interesting that they should have turned to the chinchilla for experimental work. In this condition one had a pseudo-membraneous-entero-colitis and they had induced this disease in antibiotic treated chinchillas by oral administration of cultures of staphylococcal aureous, but in a later experiment they had been able to do this with a toxin only isolated from the staphylococci. They had also reproduced this disease in cats.

On the whole subject of chinchillas, he thought that in the next few years they would see what was going to happen. There were those who believed that the chinchilla had come to stay, and there were others who believed that it was a "flash in the pan". In any case, he felt that a more natural form of husbandry combined with greater freedom and fresh herbiage was the aim which practitioners should have with their clients. He thought that a lot of "hooey" had been written by chinchillas, and particularly in regard to diet. There had been arguments about pellets and on whether rat pellets and rabbit pellets could be fed to them. He felt that the whole matter was in a state of flux.

He made no reference in the paper to virus diseases. This was an obvious omission. It was more than likely that, if one could go on histological findings in the U.S.A., which he doubted, the chinchilla could suffer from the L.C.M. viruses; but one trouble in experimental work with the chinchilla (and he was sure that it applied to the mink) was that the very expense of the animal precluded, generally speaking, its use as an experiment animal. While they might use the guinea pig, the rat and the mouse for comparable reasons, they really had to go to the chinchilla to prove these points, and without the necessary funds being forthcoming this was very difficult.

The Opener

Mr. R. N. Twisleton-Wykeham-Fiennes, who opened the discussion, said that it with the greatest pleasure that he had received the invitation to open the discussion on the two splendid papers which they had heard this afternoon, the one a discourse on diseases of mink and the other on diseases of chinchillas. He felt the more pleased because, unlike the authors, he had the advantage of being able to speak about both of these animals—both reared for the same purpose, the production of high quality furs. In all other respects the animals were utterly dissimilar; the mink was a savage hunter, a carnivore; the chinchilla, perhaps the most timid of all hunted species, was a rodent. Both these animals were so unlike in their habits, in their physiology and in almost all respects that they perhaps more than any other two animals typified the great diversity of knowledge and experience which was required of members of the veterinary profession.

The contrast between those two animals was interesting in another respect. Five to 6 years ago mink farming had been seriously started in this country. Breeding stock had been imported across the Atlantic and mink farms had begun to be established. Losses had been high from a great variety of causes, most of which had been little understood. There had been no lack of pessimists who had said that this country was unsuitable for mink, that the industry could never be established, and that even if those animals could be kept alive it would be impossible to get them to breed properly, and that in any case the pelts would be valueless. Today the British fur industry was buying 80,000 mink pelts annually, and he was assured by a well-known merchant in skins and furs that the British skins were the best on the market. In addition, they had just heard from Mr. Head of the industry's dollar-earning capacity.

The chinchilla today stood where the mink had stood 6 years ago. Large numbers of these little rodents were being imported into the country, many courageous people had invested their money into buying breeding stock, and many had taken hard knocks because of the high losses. The prices paid for these animals at anything up to £350 per pair were very high, and it was a challenge to the veterinary profession to study and overcome their diseases and so enable the breeders to emulate their forerunners in the mink trade.

It was now some 12 months since their assistance at the London Zoo had come to be requested in large degree over the diagnosis of diseases in chinchillas. From that time until the present there had been a steady weekly stream of these animals presented for *post-mortem* examination. Like other workers, they had had little precise knowledge of the diseases from which chinchillas suffered, although these animals had been kept at Regent's Park for a number of years. Like other workers, therefore, they too had had to start from scratch, perfect their methods of diagnosis and discover what were the most effective treatments to be given in each kind of case. This subject was so new that he did not wish to waste time by criticising or developing what had been said before, but rather to amplify it by giving the results of their experience and research.

Mr. Bowden had mentioned the question of staphylococcal entero-colitis, and of course this was a danger of the use of antibiotics. He thought that Mr. Bowden had correctly described Proteus as a pathogen. It was so described in chimpanzees and other animals, particularly *P. morgani,* as being the cause of diorrhoea symptoms, and it was one of the recognised causes of infantile diorrhoea in human beings. *Pseudomonas aeruginosa* was undoubtedly a pathogen in chinchillas and in many other of the animals that they dealt with in zoological collections. It was frequently present in the gut. In chinchillas it caused not only septicaemia but pneumonia as well. Giardia, in his experience, if it was not acting as a pathogen in chinchillas, would sooner or later do so. It was a deadly little protozoan, and they had found it in, if not unweaned chinchillas, at any rate in chinchillas so young that they had not fed for more than a few days. Sooner or later it was going to flare up and cause heavy losses.

In the early days, it had been found that chinchillas were dying of many varied causes, and it had appeared as if this was an unduly delicate little animal for which there was little future. A year later, he was happy to say that one could express quite different views. He regarded the chinchillas as a hardy animal likely to live and breed successfully once his own foibles and idiosyncrasies were better understood. He must, however, stress what he had said on other occasions, that this little creature was one of the most timid animals in creation. It was unduly sensitive and prone to shock of all kinds and to "stress" syndromes, and in his opinion shock or stress was a major component of the disease picture in 9 out of 10 chinchillas which died.

The shock or stress could take various forms. Sometimes the fur fell out. Sometimes death occurred from pure shock without other cause of any kind. Sometimes abortion took place or fœtuses died and were absorbed; then the death of the mother might occur some months later from septic metritis or thrombosis of the uterine veins. Sometimes latent disease such as giardiosis, salmonellosis or other infections might flare up, become septicaemic or in other ways acute, and cause the death of the host. In yet other cases, gastric or duodenal ulcer developed as a result of an anxiety neurosis and death might occur either from haemorrhage

from an ulcer or from chronic gastritis or enteritis. In the case of physical or organic disease, as with many of the animals with which they dealt at the London Zoo—gibbons, baboons, galagoes, hares, wallabies and many more—shock was a definite component of the disease picture, even where this was primarily due to ascertainable organic causes such as pneumonia or tuberculosis. This explained why sick chinchillas showed similar symptoms, even when affected with different diseases.

The chinchillas most susceptible to shock and anxiety conditions were those which were newly imported, which had not settled down to new quarters, or had been transferred to new ownership. Amongst chinchilla herds which were well established, accustomed to the food they were given and fond of their keepers, losses were surprisingly small. In these, the diseases which were deadly in the newly imported animals and in those which had not settled down, did not occur to anything like the same extent. Especially was this so with owners who had a natural sympathy for these charming and affectionate little animals. Owners should, therefore, be urged to get their herds self-contained as soon as they possibly could, and to the greatest possible extent.

Where disease occurred, diagnosis without laboratory aids was of extreme difficulty because the true symptoms from which the animals suffered was so often masked by the "shock" component of the disease. Laboratory aid was, therefore, usually necessary in order to identify infecting organisms and to select by sensitivity testing an antibiotic that would be effective. The main infectious diseases of chinchillas had been adequately dealt with by the author, and he would therefore confine his remarks to mentioning some troubles which had come his way and which had not been covered.

Mr. Bowden had made some remarks about the aggressiveness of the female. During May and July of this year, a number of chinchillas had been sent to him for *post-mortem* examination which had evidently been killed by the mate. These had all been young males, mostly with exhausted testes and seminal vesicles. This had made him wonder whether the practice of mating young males of 8 to 12 months with the females was a good one. Did those young males unduly worry the females, especially when with kit at the time of "breed-back" shortly after parturition, and did the female then turn on the male and kill him? Alternatively, did the female chinchilla, as in the case of the Chinese hamster, naturally attack the mate after the act of coitus? In any case, this appeared to him to be a question which required study so as to ascertain whether older males should be used or whether the males should be removed from their females following parturition. Another cause of accidental death of which he had seen a number of cases had been haemorrhage into one or both of the middle ear cavities. These were tremendously capacious in chinchillas and probably a delicate structure. Whether this accident arose as a result of a mild blow or whether it was occasioned by a sudden loud noise he did not know. He had encountered during the past summer one case of death from haemorrhage arising from the blood vessels at the base of the brain, and again the cause of this had not been clear, though there had been some evidence of a blow at the base of the neck. One case also had come his way in which death appeared to have been due to heat stroke. Other accident deaths sometimes happened as a result of an inexperienced owner holding a chinchilla in the wrong way. These animals were so strong and could struggle so powerfully that sometimes an owner would crush the thorax causing fractures of the ribs or hepatic haemorrhages.

It appeared to him that they could truthfully say that control has been obtained of the main killing diseases of chinchillas. There was still, however, much which required to be accomplished. Breeding records were not good in most cases, and pelts were not reaching the standard which should be achieved, if the industry was to be set on its feet. There were probably two causes for this. In the first place, there seemed little doubt that animals of poor grade had been sent in numbers to this country by some unscrupulous shippers. To take a simple example of this, the number of newly imported animals presented for advice which were suffering or had died as an indirect result of malocclusion of the teeth ("slobbers") were far too high. Many of those came with veterinary certificates, and if received on this side of the Atlantic the importers should be advised to refuse acceptance of the animals as being affected with a serious unsoundness. It was reasonable to suppose that they might also be coming with genetical unsoundnesses as well.

In regard to breeding and fur production, there was little doubt in his mind that apart from the poor genetical strains which were received, the clue lay in the role of nutrition. Chinchillas appeared to be able to eat a very varied vegetarian diet, but certain additives were essential. They had to think in terms of essential fatty acids. They knew from the case of mink how essential were methionine and choline for proper functioning of the liver. He was hoping that Mr. Dall would have something to say about coprophagy in chinchillas. They knew that, in the absence of essential fatty acids, yellow fat condition would develop, or more seriously Aleutian disease. Xanthomatosis was common also in chinchillas and a number of these animals were found with yellow fat and yellow ears indicating definite dietary deficiencies. He had little doubt that "fur slip" and fur chewing, except in so far as they were attributable to nervous conditions, were also associated with a lack of essential dietary elements.

He wanted express his thanks to the two authors for their interesting papers. He wanted to offer an apology to Mr. Head, if apology was needed, for saying so little about mink, but the paper was so comprehensive and so excellent in every way that he had felt that there was little to be said about it and that, if there was anything to be said, he was probably not the person to say it. They were all, he was sure, grateful to Mr. Head for teaching them a little about this subject. Thus the reason for not developing the theme of Mr. Head's paper was a compliment to the paper, and not the reverse.

The Chairman, Mr. J. A. Dall, then asked the meeting whether anyone had noted the fact that chinchilla exhibited corprophagia to a certain extent. Several of his clients had observed this action, and it would be valuable if other mombers of the profession who had first hand knowledge would report authentic cases.

The General Discussion

Mr. Oliver Graham-Jones (Zoological Society of London): "It is the first duty of all here present to congratulate both the authors and the opener upon the papers which they have just presented to us. It must be clear to everyone that they have done an enormous amount of work and research to list to us all the diseases which can be found upon *post-mortem* examination of dead mink and chinchillas. Indeed, they have presented us with such a comprehensive catalogue of diseases that one wonders whether the papers would not have been better addressed to a meeting of pathologists and bacteriologists. Certainly, the material is of such a high standard that it might well be published in a journal covering these subjects rather than in one covering clinical veterinary medicine. I must hasten to make it quite plain that these remarks do not in any way constitute a criticism of the papers or, indeed, of the authors. It may be an observation, however, that perhaps the British Veterinary Association has not made is clear enough to the authors that they are an Association consisting chiefly of veterinary surgeons engaged in clinical veterinary practice. This being the case, it may be that people present today would have preferred to hear far more advice about the handling, housing, travel, drug dosage, etc., as it applies to these exotic animals, the mink and the chinchilla. Pathologists, as a race, seem to be rather imaginative men, and are most inclined when they are examining a dead body to build up a clinical history around the *post-mortem* symptoms and then issue the whole thing as an authorative statement on the diagnosis and treatment of disease in the live animal. It is a truism that it is easier to be wise after the event.

"One feels that frequently in papers today there is far too much emphasis on actual disease conditions and insufficient on the maintenance of health of the animal in question and the prevention of disease. As far as chinchillas and mink are concerned, it is much more important to maintain their health and prevent them from becoming ill

than it is to attempt to diagnose disease and to treat it when it occurs. Equally, it should always be remembered that disease in these species mostly occurs when, as has been described by one of the authors, opportunists meet with lowered resistance consequent upon unsatisfactory conditions of physical well being.

"I, too, am going to confine my remarks to the chinchilla. I have two main reasons for doing this:

1. Because mink are an established fancy now, and at any rate are carnivores. Their diseases are very well catalogued and comparatively well understood.
2. I believe that because chinchillas are new to this country there is more need for remarks to be made about them. Chinchillas are, of course, rodents and thus have a different background to carnivores. Equally, the number of chinchilla owners in this country is far greater, although the *per capita* number is probably less than mink.

"Any experience which I have of chinchilla has been gained during the previous 8 years that I have been an employee of the Zoological Society of London. It is only fair to say that the chinchillas which the Zoological Society owns have always maintained exceptionally good health. They have not suffered from any of the diseases which appear to be prevalent in recently imported animals. Equally, they have never been regarded as particularly delicate animals and are treated like ordinary normal members of the rodent family. They have been kept outdoors and have been subjected to all weather conditions prevailing at the time. Thus they have been through hot weather to extreme cold weather, wind and rain, and have certainly been subjected to several London smogs. On the whole their health has been very good and any death that has occurred has been mostly due to casualties such as rats breaking in and killing stock or similar tragedies of that kind.

"Before I consider factors which influence the health and well being of chinchillas, it is necessary to consider some conditions which are responsible for lowered resistance. First of all we have travel. It is undoubtedly true that the majority of chinchillas that are imported into this country have had at least 5 journeys, sometimes in as many days. There is the journey from the ranch in Canada or America to a rail head. From there, the animals are transported to an aerodrome. They may spend a time in a staging camp here awaiting an aircraft, and then fly to Europe. Sometimes they come direct to the United Kingdom but quite often they go to the Continent first. After arrival in the United Kingdom the animals will be transferred from the aerodrome to a railway station where they will travel to a main agent. From the main agent, they will be transported yet again, perhaps by road, to the sub-agent. The sub-agent then sends the animal to the final owner which involves a further journey. During this time, the animal has been subjected to a wide variety of temperatures, air pressures and diets, but even more important, perhaps, have been subjected to a cacophany of varying noises coupled with rapid changes in personalities of those people handling it.

"Secondly, we must consider chinchilla diet. In my opinion much of the current diet is a false diet and has been developed as the result of observation of those foods fed to laboratory animals. I do not believe it necessary to consider the chinchilla as a laboratory animal and I think it is far more favourable to regard it as a rodent that is capable of eating, digesting, and thriving upon ordianry rodent diet. I think artificial diets like pellets are probably bad for teeth and digestive tract and they may well stimulate or encourage intestinal bacterial infections. It is true, indeed, that some pellet feeding can cause impaction by lack of gut tone.

"Many of the types of housing currently popular for chinchillas do not fit in any way the animal background of natural history. In the wild, as is well known, the chinchilla comes from areas in South America high up in the Andes at altitudes of perhaps over 10,000 ft. There is plenty of space to run around there and the animal has in the main only two preditors, namely the birds of prey and the wild fox. In captivity, therefore, the size of cage available should be as large as possible and this has an important bearing upon certain physiological relationships of groups. The temperature at which chinchillas are frequently kept in this country is ridiculously high. Ideally the animals should be kept outside since they are very resistant to variation and change, providing they have somewhere insulated where they can sleep. Exercising facilities are very important for chinchillas which have a tremendous background of play activity apart from anything else. The chinchilla should have somewhere of complete security where it knows it can go and cannot be attacked. The animal in the wild is not an aggressive little creature—rather the opposite—and consequently it is thrown into a state of terror if it cannot get away into an area where it knows it is absolutely secure.

"*Noise.* If you have ever examined the extent of the auditory bullae of the chinchilla skull you will note how vast they are in comparison to the rest of the skull area: perhaps it is the largest proportion of auditory apparatus to skull of any small mammal. The development of the hearing apparatus is one of Nature's ways of protecting the defenceless creature against predators. This exaggerated hearing apparatus in the chinchilla can be the cause of its downfall in captivity, since any small noise is exaggerated beyond that of many other animals. This very sensitivity can lead to internal haemorrhages in the ears either from an excessive noise, or, indeed, from shock and great exertion.

"*Handling the chinchilla.* The owner should be warned of the dangers of rough handling. There are those people who advocate picking the chinchilla up by the tail. I have no doubt that this is quite a safe procedure for the experienced handler but it can be hazardous to the ignorant. One must remember that the tail of the chinchilla is not just a decorative organ. It has certain physiological importances which should not be forgotten. It is, in fact, part of the animals' defence apparatus and can be used as camouflage. Equally it is part of the temperature maintaining apparatus and in any case it is extremely sensitive. Rough handling of the tail can take away the animal's sense of security.

"*Natural history and care in captivity.* At the present moment there is a tremendous amount of ignorance amongst chinchilla owners of a lot of the natural history and background to the chinchilla, and it is the veterinary surgeons job to introduce the owner to these facts. Many of the people handling chinchillas in large numbers, in other words dealers, are inclined to classify themselves as experts and to pass on their inaccurate information to the new owner.

"*The shock syndrome.* We are all paying a lot of attention these days to the presence of stress and the results of the shock syndrome. There are very few animals which show these effects to a greater degree than the chinchilla, and there are many case histories of an animal which has shown no actual clinical symptoms of illness but which is found dead a few hours after having apparently been perfectly healthy. These deaths are undoubtedly due to the cumulative effect of various stress factors which have produced delay and chronic shock state. Careful examination of the carcass will show all sorts of symptoms such as nutmeg liver, exhausted adrenals, collapsed lungs, fluid in the chest, etc., which suggest the shock syndrome.

"*Diseases.* In the main, I have found that the commonest diseases of chinchilla are in 3 main groups:
(1) Digestive; (2) Bacterial; and (3) Skin diseases.

1. *Digestive diseases.* (a) Frank indigestion. The bacterial invasion of the intestines or (b) protozoan infections such as giardoisis. (c) Imbalances and deficiencies of diet.
2. *Bacterial diseases.* These are chiefly chest diseases which are rapid and lead inevitably to pneumonia.
3. *Skin diseases.* Fur slip is a complicated syndrome but is probably in fact due to shock and/or deficiencies. Fungus disease of the skin (epidermophyton) is recognised.

"In examining the chinchilla for health, the following points should be noted. The animal should be healthy in appearance. That is to say the fur should be 'alert' and even throughout the body, the ears should be upright and twitching slightly all the time. The tail should be brisked up and carried backwards over the body. The eyes should be well pronounced in the head and shining. The virbrissae should be well extended and eager. The animal should be playful and inquisitive, and should not lurk in the background except during its sleeping period.

"A careful examination of dentition should always be made. The prominent rodent incisors should be clearly chiselled off at their angles to enable the animal to bite off its food properly. The dorsal incisors should somewhat overhang the ventral incisors but it should be possible for the animals teeth to meet in a chiselling motion. The molars should be examined using a canine aureoscope. Any inclination of the molars to bow laterally with the production of a sharp lateral edge should be regarded with suspicion: this usually indicates malocclusion, a dental condition which in itself can lead to slobbers at best, and to chronic indigestion at worst. Such a chinchilla should never be allowed to breed.

"In conclusion, Mr. Chairman, I should like to repeat my congratulations to the authors of these papers, and to Mr. Fiennes, the opener."

The Replies

Mr. K. W. Head, in reply to the discussion, made the following comments. In answer to Mr. Jones' point that the paper should have concentrated more on treatment, he said he knew there were at least half a dozen veterinary surgeons in general practice who kept mink and who, he had hoped, would have contributed their experiences to the discussion on this aspect. On the question of vaccination, the Trade Exhibition had pamphlets on vaccination for distemper and virus enteritis.

To administer drugs as part of a disease control programme or in the outbreak of a disease in which the mink were still eating their full ration, the dose of the drug for all animals on the farm was calculated, then dissolved in water if necessary, added to the ration; this was then mixed thoroughly, so that each mink received the correct dose. This method minimised trauma during handling for dosing and also reduced the risk of escape. As an example, he mentioned the use of 1/28th of an ounce of ammonium chloride per mink per day throughout the summer months as a control for urinary calculi formation. Similarly, since most mink farmers incorporated either Aureomycin or Terramycin feed supplements in the ration, if the mink developed non-specific enteritis, the level of antibiotic feeding should be trebled and, in stubborn cases, the best way was to change over to the other antibiotic feed supplement. The sulpha drugs and nitrofurans could also be administered in the diet in this way. In acute diseases, or diseases in which the mink went off their feed, or where the drug of choice rendered the food unpalatable, the theapeutic agent should be given by intramuscular injection into the hind limb or by subcutaneous injection into the skin flap behind the shoulder.

Miss Hynd had asked about the risks of distemper to a mink farmer. He thought that most pathologists would agree with him that diagnosis of distemper in any species was extremely difficult. He did not think that the profession had enough experience to be dogmatic about the behaviour of a distemper outbreak in susceptible animals kept on this scale, e.g., how many veterinary surgeons had treated a distemper outbreak in a kennels of 1,500 dogs? So he could not say how serious it would be in every case but in Scandinavia, where distemper outbreaks had occurred in non-vaccinated animals, it could have a very high morbidity and mortality, both as an acute disease and with chronic deaths some months afterwards.

As to how to advise a client, he thought that it depended on where the mink farm was. In the middle of the moors, with no dogs for miles, it might be more economical to insure against getting distemper rather than pay the veterinary surgeon's bill for vaccination. Near a town with an increased risk of exposure to the virus, the reverse was probably true, since the insurance premiums would be much higher and the risk to high quality breeding strains greater.

Mr. R. S. T. Bowden, in reply to the discussion, thanked Mr. Twisleton-Wykeham-Fiennes for his opening remarks. He had no comment to make on those opening remarks, except to say that he had been very pleased to hear the emphasis placed on shock. From his own point of view, he could hardly draw too much attention to shock, because sometimes practitioners were not particularly pleased when one wrote back to them and said that an animal died from shock—they might be inclined to ask, And what else?" But these animals were very susceptible to shock.

Was there any evidence that chinchillas required vitamin C? He had no actual evidence, but he imagined that as an herbivorous animal vitamin C would be very much required. Could Giardia be diagnosed in dead or decomposed or semi-decomposed carcases? Yes, certainly.

On the question of salvaging pelts, he had not very much experience, but generally he would say that in the case of any animal that had died of disease the pelt was pretty useless. Certainly the hairs fell out, and could be plucked out very easily.

He agreed with Mr. Jones that the paper could have had a greater clinical bias. He had hoped, of course, that some of the clinical conditions would have been referred to in the discussion. He suggested that at a future Congress a clinical paper on mink and chinchillas would be very welcome.

He also agreed with Mr. Jones about diet. He felt, as he had already said, that a lot of nonsense had been written concerning diet in the chinchilla, and he felt that they had to approach this from a more natural angle. He also agreed that the temperature at which the animals were kept was often unnatural, and that the nutritional requirements, from a pellet point of view, were not yet known. He did not think that they should turn their backs on pellet feeding, but he felt that this was not yet properly worked out.

He agreed with Dr. Cotchin that if one was going to use chinchillas, research was going to be an expensive business. This was a matter which the profession must have regard to: it was something on which he hoped the industry would approach the profession. It was a subject that had got to be got down to if the industry was to continue. The research could not be done without funds, for the research could not be done without the live chinchillas.

Mr. R. N. Twisleton-Wykeham-Fiennes, replying to some of the points that had been raised on his opening contribution, said that vitamin C was essential to the chinchilla. On the subject of the pelt, the fur of the chinchilla was extra-ordinarily sensitive. If the chinchilla was frightened, its fur would come out. If it was scared when it was killed. the pelt would shed the fur. The chinchilla must be killed in a way in which it did not know that it was being killed. It must not be frightened, otherwise its fur would slip after death.

Miss Brancker had referred to sudden death. He did not think that the sudden death in chinchillas was due to the fact that they concealed sickness, as so many wild animals did—for instance, sheep. He thought it was due to the fact that they went into the state of shock very quickly. Another cause was that where there was anything wrong with the gut, the gut went into spasm very easily. With animals that had died suddenly—perhaps with a history of diorrhoea just before death—one found sometimes a paralysis of the lower gut.

The Chairman proposed a vote of thanks to the authors of the two papers, the opener of the discussion, and all those who had taken part in it.

Some Tumours of Dogs and Cats of Comparative Veterinary and Human Interest

BY

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SUMMARY—A survey is made of 4,187 tumours from dogs and 571 tumours from cats that have been examined in the Department of Pathology, Royal Veterinary College, in the period 1940-58 inclusive. The rarity of cancer of the lung, stomach, large intestine or uterus in dogs and cats contrasts with their importance in human beings, while, on the other hand, some tumours that are common in dogs and cats but rare in man would appear to provide suitable material for detailed study, such as mast-cell tumours of the skin of dogs, mixed mammary tumours of the bitch, Sertoli-cell tumours of the testis of dogs and lymphosarcoma in cats. The study of spontaneous tumours in dogs and cats might be developed particularly in two ways: first, by histological, histogenetic and other analyses of the tumours themselves and, secondly, by therapeutic studies.

IN this paper a brief survey is made of tumours of dogs and cats encountered in the College here, highlighting examples from two aspects: from the veterinary aspect, features of certain common tumours are summarised, and attention is drawn to some examples of uncommon tumours, while from the comparative aspect reference is made to certain tumours that may interest medical pathologists and cancer research workers. Finally, some implications of the observations are considered.

Material Examined

This survey is based on 2,361 tumours of dogs (Cotchin, 1954a) and 464 tumours of cats (Cotchin, 1957) examined here since 1940 and previously reported, supplemented by a further 1,826 tumours from dogs and 107 tumours from cats examined up to the end of 1958. (Reference should be made for further details to the earlier papers and also to Cotchin, 1951, 1954b and 1956.)

Source of Material

Some of the tumours examined have been from animals coming into the College for operation or autopsy, while others have come in from practices as biopsy, operation or post-mortem specimens. This material is of a selected kind, having to pass two filters—the owner and the veterinary surgeon.

Observations

Age Incidence of Tumours in Dogs and Cats. The general rule that neoplasms occur with greater frequency with advancing age applies to dogs and cats (Table I) as well as to man.

The dogs and cats with tumours are clearly in general a good deal older than the run of dogs and cats coming into the College clinic with other conditions. However, the veterinary surgeon should not avoid making a diagnosis of malignant neoplasm in a young animal merely on account of its youth, as the following examples will show.

Malignant Tumours in Young Animals

(a) Under One Year of age

(i) Dogs

There were two malignant kidney tumours, and lymphatic leukosis (so-called "pseudo-Hodgkin's disease") was seen in two 4-month-old puppies. Four young dogs (5, 5, 6 and 8 months old respectively) showed a particular kind of gum tumour, locally malignant but radio-sensitive, which has been called a "papilliferous epithelioma".

(ii) Cats

The four animals under one year of age with malignant tumours were affected with lymphosarcoma: of the thymus region of an 8-month-old castrated male and of a 9-month-old female; of the small intestine of a castrated male; and (in the generalised form of lymphatic leukosis) in a 9-month-old Siamese cat.

(b) From 12 to 24 Months of Age

(i) Dogs

Lymphosarcoma again was often seen: there were four cases of lymphatic leukosis, while five other dogs

TABLE I

SHOWING THE AGE DISTRIBUTION OF TUMOUR-BEARING DOGS AND CATS, COMPARED WITH OTHER CLINICAL CASES

Age (years)	DOG				CAT			
	2,629 tumour-bearing dogs		2,500 clinic dogs		470 tumour-bearing cats		600 clinic cats	
	Number	%	Number	%	Number	%	Number	%
Under 1	35	1·3	680	27·2	4	0·9	181	30·2
1–5	474	18·0	1,264	50·6	69	14·7	270	45·0
6–10	1,478	56·2	426	17·0	215	45·7	113	18·8
11–15	626	23·8	130	5·2	155	33·0	35	5·8
Over 15	16	0·6	0	0·0	27	5·7	1	0·2

showed localised lymphosarcoma. There was a further example of "papilliferous epithelioma" of the gum.

A tumour now coming into prominence was the osteosarcoma: there were four examples of osteosarcoma of the rib and three of osteosarcoma of the humerus. Other malignant tumours were a sarcoma of the urinary bladder and two examples of sarcoma of the palate.

(*ii*) *Cats*

There were no examples of malignant tumour in cats in this age group.

(c) From 2 to 4 Years of Age

There were again a number of cases of lymphosarcoma in dogs and cats and of osteosarcoma in dogs but, while these were the predominant malignant tumours, others were seen.

(*i*) *Dogs*

In two 2-year-old dogs there were a carcinoma of the tonsil and a carcinoma of the pancreas. Five 3-year-old dogs showed carcinomas—of the mammary gland of a bitch, of the intestine of two male mongrels, and of the frontal sinus and of the lung respectively of two male Alsatians. There was also a malignant melanoma of the eye of a 3-year-old male mongrel. (In passing, it may be noted that testis tumours were seen in three young Pekingese dogs.)

(*ii*) *Cats*

A probable carcinoma of the intestine was seen in a 2-year-old.

Malignant Tumours in Old Animals

While the bulk of tumours occur in middle-aged animals, we have, turning to really old animals, examples of malignant tumours as follows:

(*i*) *Dogs*

There were seen malignant melanoma of the palate and the gum of a 17-year-old dog and, in three 16-year-old dogs, melanoma of the gum, squamous-cell carcinoma of the digit, and sarcoma of the hard palate.

(*ii*) *Cats*

We have examples of leiomyosarcoma of the kidney of a 22-year-old cat, humerus osteosarcoma in a 20-year-old, ceruminous adenocarcinoma of a 19-year-old, oesophageal carcinoma in a 17-year-old, and, in six 16-year-olds, carcinomas of the tongue, mammary gland, gum, oesophagus and skin, and lymphosarcoma of the intestine.

Breed Incidence of Tumours in Dogs

There are examples of apparent breed susceptibility to different kinds of tumour that might be mentioned: thus the Boxer is liable to mast-cell tumours and the Great Dane to bone sarcomas (Table II).

TABLE II

SHOWING SYSTEMS OF ORIGIN OF 98 TUMOURS FROM BOXERS AND 23 TUMOURS FROM GREAT DANES

System of Origin	Number of tumours	
	Boxers	Great Danes
Skin	56 (incl. 27 mast-cell tumours)	2
Female genital	7	3
Alimentary	9	4
Male genital	9	1
Skeletal	6	11
Lymphatic	7	1
Others	4	1
	98	23

Sex Incidence of Tumours in Dogs and Cats

Taking all tumours together, the number of females in our series of tumour-bearing dogs and cats is higher than in the series of "clinical cases", but, if tumours of the genital system (including mammary gland) are omitted, the proportions of males and females are similar in the tumour-bearing and the clinic groups (Table III).

TABLE III

SHOWING SEX OF 2,686 TUMOUR-BEARING DOGS, AND 435 TUMOUR-BEARING CATS, COMPARED WITH THE SEX OF 2,500 DOGS AND 600 CATS EXAMINED IN THE CLINIC

	DOG		CAT	
	Male %	Female %	Male %	Female %
Total tumour-bearers	46	54	69	31
Excluding animals with "genital system" tumours	62	38	82	18
Clinic cases	59	41	79	21

For certain tumours, however, there is a preponderance of males, e.g., the circumanal adenoma of the dog, and probably carcinoma of the upper alimentary tract of the cat.

TABLE IV

SHOWING SYSTEMS OF ORIGIN OF 4,187 TUMOURS OF DOGS AND 571 TUMOURS OF CATS, EXAMINED IN THE PERIOD 1940–58 INCLUSIVE

System of origin	DOG			CAT		
	Grand total	%	Ranking order	Grand total	%	Ranking order
Skin	1,572	37·5	1	140	24·5	2
Female genital*	1,086	25·9	2	68	11·9	4
Alimentary	588	14·0	3	164	28·7	1
Male genital	299	7·1	4	0	0·0	–
Skeletal	164	3·9	5	47	8·2	5
Lymphatic	159	3·8	6	70	12·3	3
Miscellaneous	319	7·6	–	82	14·4	–
	4,187			571		

* Including mammary gland.

System of Origin of Tumours of Dogs and Cats

In Table IV the series of tumours previously reported and the more recently examined specimens have been combined to give a grand total of tumours examined here in the period 1940-1958. The information about system of origin is likely to be correct in most cases but the possibility of error increases in further classification, both as to histological type and to clinical behaviour (Cotchin 1951).

The ranking order of these systems is not the same for the dog and the cat: in the former, the skin has provided more tumours than any other system, while in the cat, first place is held by the alimentary system.

For the cat, it is possible to classify the malignant tumours simply as carcinomas or sarcomas (Table V), but this is not feasible for the more complex dog tumours.

TABLE V

SHOWING SYSTEMS OF ORIGIN OF 407 MALIGNANT TUMOURS OF CATS

System	Carcinoma	Sarcoma	Total
Alimentary	97	54	151
Lymphatic	—	67	67
Cutaneous	23	35	58
Genital	48	2	50
Skeletal	—	45	45
Urinary	1	19	20
Respiratory	7	4	11
Eye	3	1	4
Endocrine	1	0	1
	180	227	407

1. Tumours of the Skin and Associated Structures

(a) Dog

The tumours in this series can be classified into 3 main groups—those of epithelial or of connective tissue origin, and melanotic tumours—with a separate group of miscellaneous and undiagnosed tumours (Table VI).

TABLE VI

CLASSIFICATION OF 1,572 NEOPLASMS OF THE SKIN OF DOGS

Type	Number	Per cent.
Epithelial origin	**515**	32·8
Basal cell and glandular (including 167 hepatoid adenomas)	395	
Squamous-cell carcinoma	76	
Epidermal cyst	26	
Papilloma	18	
Connective tissue origin	**630**	40·1
Sarcoma (including 91 mast-cell tumours)	405	
Fibroma	130	
Lipoma	52	
Angioma	43	
Melanoma	**254**	16·1
Miscellaneous and undiagnosed	**173**	11·0
	1,572	100·0

The tumours that are perhaps of most interest in this series are the hepatoid and other glandular tumours, mast-cell sarcomas and melanomas.

(i) Hepatoid and Other Glandular Tumours of the Skin

Four out of five (395/515) of the skin tumours of epithelial origin were of basal-cell or glandular type, including baso-squamous tumours with sebaceous differentiation, so-called sweat gland tumours (see Groth, 1955) and hepatoid adenomas. The last-named tumours occur only in dogs. They are usually seen as so-called circumanal adenomas, but they also occur by the side of the prepuce, on the ventral side of the root of the tail and on other parts of the skin, chiefly on the back. These tumours arise from circumanal and other modified sebaceous glands which, from their supposed method of secretion in intercellular canaliculi, were called "hepatoid" glands by Schaffer (Cotchin, 1947). The tumours may reach a large size, particularly in extra-anal positions. They occur almost exclusively in old male dogs. These tumours, which are usually sessile, may ulcerate and bleed, and may be multiple; they show a grey or fawn-coloured, smooth, faintly lobulated cut surface. They rarely metastasise (there was one possible malignant case in our series) but they may recur locally.

(ii) Mast-cell Tumours

Partly because of the popularity of Boxers, these tumours are now commonly encountered. The ages of our affected dogs range from 2 to 13 years (average 8). Of 88 affected animals, at least 27 were Boxers, 10 were Bull Terriers and 2 were Bulldogs. In 7 cases the lesions recurred after operative removal and 7 dogs showed metastases—6 in the regional lymph node, the seventh in the lungs and elsewhere.

These mast-cell tumours vary in size from 1 cm. to 12 cm. across. They tend to be oval in shape and to lie as rather flattened bulges of the skin, which may be ulcerated. They grow in the dermis and subcutis, the cut surface being variably fibrous, with patchy light-brown areas scattered in flabby fibrous tissue. The histology is variable.

Bloom, Larsson and Äberg (1958) reported that some animals with mast-cell tumours bled profusely from the surgical wound following operative removal, the haemorrhage beginning generally 5 to 24 hours after operation. (No history of this kind has been given in our cases.) It was found that the haemorrhage could be arrested, generally within 15 minutes, by the administration of anti-histamine. McClelland (1957) found some tumours to respond well to X-ray therapy.

(iii) Melanomas

Melanomas are common tumours of the skin of dogs. A series of 83 skin melanomas of dogs has been described in detail (Cotchin, 1955). In a more recent series of skin tumours, 90 further melanomas have been examined. Spaniels (21), Scottish Terriers (19) and Airedales (9) were the most numerous breeds, providing over half the tumours. Of these 90 tumours, 9 showed metastases. The digit was a common site and the scrotum and prepuce were also quite often affected.

A further tumour of the subcutis of the dog which we are increasingly recognising is the so-called haemangiopericytoma, as described by Mulligan (1955) and Yost and Jones (1958). This tumour, which is perhaps derived from the pericytes of Zimmermann, occurs principally in the subcutis of older dogs. The neoplasms are usually firm, whitish, lobular and

attached to deep tissue below movable skin. Metastases are not often seen but the tumours may recur.

These tumours may be confused with tumours of fibrous, smooth muscle or nerve-sheath origin, and it is probable that a number of the tumours classified above as sarcomas, or as fibromas, would be found on re-examination to be of this type.

(b) Cat

Many of the skin tumours of cats are baso-squamous pigmented structures, of firm texture and well-defined edge, which do not often metastasise but may recur locally. Particularly interesting, in our series of 23 carcinomas, were the squamous-cell carcinomas (of which 6 out of 13 occurred on the ear-flap) and the ceruminous gland adenocarcinomas. Tumours of the ceruminous glands are rare in man, and are only occasionally seen in dogs (we have three examples of mixed ceruminous tumours resembling mixed mammary tumours), but in the cat definitely malignant tumours of these glands are occasionally seen, with widespread lymph-nodal and visceral metastasis, and other suspected malignant tumours are not very rare. No definite mast-cell tumours were recognised in our series of feline skin tumours but they do occasionally occur (Meier, 1957b; Head, 1958).

2. Female Genital System

(a) Dog

The important tumours of the female genital system of the dog are those affecting the mammary gland (Table VII).

TABLE VII
SITE OF ORIGIN OF 1,086 TUMOURS OF THE GENITAL SYSTEM OF THE BITCH

Site of origin	Number	Per cent.
Mammary gland	895	82·4
Vagina	123	11·3
Ovary	51	4·7
Uterus	11	1·0
Cervix	4	0·4
Ovarian bursa	2	0·2
	1,086	100·0

These mammary tumours form an extraordinarily interesting group. Details of a study of 424 tumours have been published (Cotchin, 1958a). Since that report, a further 281 mammary tumours from bitches have been examined, of which 143 were classified as benign and 138 as malignant: of the latter, 21 showed metastases.

(b) Cat

In the cat the bulk of the tumours of the female genital system are mammary tumours (52/68) and in our experience most of these (45/52) are adenocarcinomas. Mammary tumours tend to occur in old cats and they are mostly in the anterior glands. They are usually seen in entire females, but are not restricted to them. Mixed mammary tumours must be rare in cats, if they occur at all.

Uterus

Carcinoma of the uterus is a rare condition in dogs and cats. In our series there is no example of uterine or cervical carcinoma in the dog and only one example of a uterine carcinoma in a cat.

Vagina

The predominant tumour of the vagina of the bitch is composed of fibrous tissue or smooth muscle, or of a variable mixture of both. Some of the lesions are more hyperplastic than neoplastic, others are well-defined, submucous, partly bulging or pedunculated masses. Their hormonal origin seems probable, as they may become obvious after oestrus. They may be multiple, a point worth noting clinically. Rather similar growths, which may be multiple, may be found in the vagina, as also in the uterus, of the cat.

Ovary

(a) Dog

TABLE VIII
SHOWING CLASSIFICATION OF 51 TUMOURS OF THE OVARY OF THE BITCH

Type	Number
Granulosa-cell tumour	21
Adenoma	10
Adenocarcinoma	10
Unspecified malignant	3
Theca-cell tumour	2
Seminoma-like (Dysgerminoma?)	2
Lipoma	1
Teratoma (Dermoid cyst)	1
Tubular adenoma (Sertoli-cell like)	1

Ovarian tumours are not very rare in the bitch, and some are important because of their apparent excessive hormonal activity, associated with persistent oestrus or with pyometra. The tumours may be bilateral in origin and very occasionally they give rise to peritoneal implantation metastases, accompanied by ascites.

One dermoid cyst is noted in Table VIII, and Riser and others (1959) have recently reported another.

(b) Cat

Ovarian neoplasms are rarely reported in cats. Baker (1956) saw a malignant granulosa-cell tumour, and we have only one example—an adenocarcinoma, affecting a 7/8-year-old cat and producing implantation metastases.

3. Tumours of the Alimentary System

Over 60 per cent. of the tumours of the alimentary system of dogs (Table IX) were in the mouth and over 20 per cent. were in the intestine.

TABLE IX
SHOWING SITES OF ORIGIN OF 588 TUMOURS OF THE ALIMENTARY SYSTEM OF DOGS

Site	Number	Per cent.
Mouth (including 117 tonsil carcinomas)	364	61·9
Oesophagus	2	0·3
Stomach	9	1·5
Intestine	130	22·1
Liver	64	10·9
Pancreas	16	2·7
Parotid gland	3	0·5
	588	99·9

In cats the important malignant tumours of the system (Table X) were carcinomas of the upper alimentary tract and sarcoma of the intestine.

TABLY X

CLASSIFICATION OF 151 MALIGNANT TUMOURS OF THE ALIMENTARY SYSTEM OF CATS

Site	Type		Total
	Carcinoma	Sarcoma	
Tongue	18	1	19
Tonsil	13	1	14
Gum, palate, pharynx ..	17	5	22
Oesophagus	21	1	22
Stomach	0	4	4
Intestine	11	42	53
Liver	6	0	6
Pancreas	9	0	9
Parotid	2	0	2
	97	54	151

(i) Upper Alimentary Tract (Mouth, Pharynx, Oesophagus)

(a) Dog

In this series there was one possible carcinoma of the oesophagus. The most interesting tumours of the mouth of the dog are tonsil carcinomas and oral melanomas.

Squamous-cell carcinoma provided 20 per cent. (117/588) of the tumours of the alimentary tract in this series, or 2·8 per cent. of the total number of tumours. The tumours tend to occur in dogs of about 8 or 9 years of age (range 2 to 14½ years). The right tonsil (37/58) was more often affected than the left (21/58). In one series of 37 affected dogs, there was in four instances histological involvement of the opposite tonsil by carcinomatous change and occasionally bilateral involvement was seen macroscopically. Metastases were commonly present within 1 to 16 weeks of the onset of symptoms (in fact, symptoms referable to the primary tumour may not be noted—one dog was presented for a suspected femur tumour, having a metastasis there, and another because of the acropachia accompanying a lung metastasis); in some dogs the presenting sign was enlargement of the regional node by metastasis. Secondary growths were present in 44 of one series of 72 affected dogs, involving chiefly the superior cervical lymph node on the same side (27 dogs), but it was also seen in the opposite node (6 dogs), the mandibular node, the prescapular node, or (in 11 of the 44 dogs) the lungs. It seems unlikely, therefore, that the treatment of tonsil cancer by local incision or by irradiation will be generally successful.

Melanomas occur in various parts of the mouth, but chiefly inside the cheek or on the gums, and the prognosis for them is very poor.

(b) Cat

There were in our series 68 squamous-cell carcinomas of the upper alimentary tract—i.e., 11·9 per cent. of the total number of tumours, or 16·7 per cent. of the total of malignant tumours. These tumours occurred particularly in old castrated males. There are certain sites of predilection: the tongue cancers occurred exclusively on the ventral side of the tongue and the oesophageal cancers were usually but not always found just within the thorax. These carcinomas, which interfere with eating or swallowing, have a characteristic white, firm but friable, cut surface.

(ii) Stomach

(a) Dog

Tumours of the stomach are rare in dogs. The 9 examples in this series comprised 3 sarcomas, 1 leiomyoma, 1 adenoma and only 4 adenocarcinomas. (Three further examples of gastric carcinoma have been seen here this year, and Sandersleben (1956) reports a carcinoma in a 9-year-old Scottish Terrier.)

(b) Cat

The 4 stomach tumours in this series were all examples of lymphosarcoma.

(iii) Intestines

(a) Dog

The important tumour of the intestine of the dog is a lymphosarcoma, although carcinomas do sometimes occur. The lymphosarcomas occur most often in the small intestine. The tumours are quite often multiple, ranging from 2 to 7 or more in number. They have a characteristic appearance: they are diffuse or annular thickenings of the wall of the gut by greyish-white, not very firm, tissue and, because of the involvement of the muscularis, they become more or less diverticulate, often with local adhesion of the great omentum. Metastases are often present—chiefly in the mesenteric node, but also in other abdominal and thoracic lymph nodes and organs.

Carcinoma of the intestine is not very common. In 9 out of 15 of our cases, the rectum was affected and in only 3 dogs were the lesions in the small intestine. The rectal carcinomas were associated with tenesmus and passage of blood-stained faeces. (See Banks, 1955; Überreiter, 1955; Liesegang, 1958.)

(b) Cat

In the intestine of the cat, the important tumours are sarcomas, usually lymphosarcomas. They nearly always occur in the small intestine. The tumours commonly metastasise; however, Patterson and Meier (1955) report excellent health of a 5½-year-old cat for 23 months after operative removal of a lymphosarcoma of the terminal ileum, caecum and colon.

Of the 11 examples of carcinoma of the intestine in this series, 10 affected the small intestine (of these, 7 had metastasised). The other carcinoma affected the caecum of an 11-year-old castrated female cat—there were metastases in the lungs and also in the tibia, the latter forming the presenting clinical sign, being thought to be a primary bone tumour.

(iv) Liver

(a) Dog

The liver tumour of the dog is commonly derived from hepatic and not from bile-duct epithelium. It usually appears as a large bulging mass, looking rather like fatty liver, and it may rupture spontaneously.

(b) Cat

The 6 malignant tumours of the liver of cats comprised 3 bile-duct carcinomas, 2 liver-cell carcinomas and 1 anaplastic carcinoma.

(v) Pancreas

(a) Dog

There were 15 adenocarcinomas of the pancreas, all apparently of exocrine origin. Such tumours tend to produce metastases in the liver and may be accompanied by signs of obstruction of the bile-duct or of the pylorus, as well as of pancreatic disturbance (Gans, 1958; Stünzi and Suter, 1958). (Reference should be made to the important article by Cello and Kennedy (1958), who report hyperinsulinism in three dogs due to pancreatic islet-cell carcinoma. Symptoms of weakness, dyspnoea, ataxia and convulsions tended to occur at the end of periods of fasting, or after exercise, and these were relieved by the administration of glucose. Diagnosis can be confirmed by demonstration of subnormal blood-glucose levels after fasting.)

(b) Cat

There were 9 cases of exocrine adenocarcinoma of the pancreas—6 in castrated males. Metastases were present in the liver in 6 cats.

4. Tumours of the Male Genital System

(a) Dog

The 299 tumours of the male genital system were almost entirely of the testis: the 29 examples of non-testicular tumours included 13 of the penis, 15 of the prostate and 1 of the spermatic cord.

A series of 268 dogs with testicular tumours were divided into three groups according to the only (or largest) tumour present: Sertoli-cell tumour in group I (135 dogs), seminoma in group II (90) and interstitial-cell tumour in group III (43). The average age of group I dogs was 8½ years; of group II, 10 years; and of group III, 11½ years. In 71 out of 165 dogs, the neoplastic testes were ectopic—in 54 dogs in group I, in 16 in group II, and in only 1 in group III. The usual site of the ectopic tumours was at or near to the external inguinal ring, but in 7 dogs (6 in group I, 1 in group II) they were intra-abdominal.

In 54 dogs (41 in group I, 4 in group II, 9 in group III) there were signs of hormonal disturbance—alopecia in 21 dogs, feminisation in 18, and both alopecia and feminisation in 15. Twenty-seven of the 33 dogs with feminisation were in group I, 2 were in group II, and 4 in group III.

Not infrequently, multiple tumours and multiple types of tumour were present in affected testes. Metastases were present in 2 dogs in group III, and probably in 2 dogs in group I (metastasising Sertoli-cell tumours in dogs are also reported by Brodey and Martin, 1958).

The 13 tumours of the penis in this series included a squamous-cell carcinoma—a rare tumour in dogs, 2 other examples being reported by Kast (1957a)—and 10 specimens of transmissible venereal tumour. This latter extraordinary lesion, the neoplastic nature of which needs confirmation, affects both dogs and bitches and is transferred venereally. It may regress spontaneously (which perhaps speaks against its truly neoplastic nature), and is radio-sensitive (as in the bitch—see the recent report, e.g., by Hataya, Shirasu and Takeuchi, 1958). This is an apparently unique case of a spontaneously transplantable tumour which does not arise *de novo* in its host, but only by transfer from another affected animal. The tumour has become very rare, if not extinct, in Great Britain, but is occasionally seen in quarantine dogs, being still common in some places abroad.

The 15 lesions of the prostate were classified as adenomas in 7 instances and carcinomas in 8: the only metastasising tumour was an anaplastic carcinoma of rather doubtful origin. In view of the common occurrence of hyperplasia of the prostate in dogs (see, e.g., Berg, 1958), the diagnosis of carcinoma of the prostate needs making with care. Grant (1957) has recently described metastasising prostatic carcinomas in 2 dogs—one, with clinical signs of C.N.S. involvement, had metastases in the brain.

(b) Cat

There were no tumours of the male genital system of cats in this series (recently, a testis tumour in an old male cat has been seen here and other tumours are reported by Meier, 1956).

5. Tumours of the Skeletal System

In the dog, the long limb bones and the ribs together provide 75 per cent. (115/154) of the bone sarcomas; in the cat, the humerus and femur together provide over half (25/45) of the bone tumours (Table XI).

TABLE XI

SHOWING SITES OF ORIGIN OF 154 TUMOURS OF BONES OF DOGS AND 45 TUMOURS OF BONES OF CATS

Site or origin	Dog	Cat
Humerus	35	15
Radius	20	3
Femur	17	10
Tibia	22	1
Rib	21	0
Other bones	39	16
	154	45

A recent article (Brodey, McGrath and Reynolds, 1959) gives a valuable account of the clinical and radiological features of a series of 75 bone tumours of skeletal origin in dogs.

6. Tumours of the Lymphatic System

(a) Dog

The series of 159 tumours of the lymphatic system of dogs includes 82 examples of lymphatic leukosis ("pseudo-Hodgkin's disease") and 20 of the 65 tumours in this system in cats were certainly (12) or probably (8) of this type. Lymphatic leukosis in dogs has recently been well described by Meier (1957a) and has also been extensively studied in this Department by Irfan (1958), whose cases (56 in dogs, 8 in cats) are omitted from the figures in this present survey.

The condition somewhat misleadingly called "pseudo-Hodgkin's disease" in dogs is in fact lymphatic leukosis, the main sign being a more or

less bilaterally symmetrical and generalised enlargement of lymph nodes by a presumably neoplastic process involving the lymphocytic series.

In Irfan's cases, the study of biopsy impression smears of enlarged lymph nodes was found to be a most useful method of diagnosis, showing almost all the cells to be lymphoblasts. Examination of blood smears was less helpful, although in 16 of 53 dogs examined Irfan found lymphoblasts (a pathognomonic feature) in the blood smear.

Irfan reports that 8 dogs treated with chlorambucil (Leukeran brand) showed a marked clinical improvement, lasting from 2 to 6 months, but all were ultimately destroyed, except one: this dog had been treated while in an early clinical stage of the disease and remained alive and active, with lymph nodes reduced in size, for over a year at least. Another dog treated with hydrochloride of tri-(2-chloroethyl)-amine (Trillekamin) remained alive for a year, and one dog treated with cortisone also showed improvement for a few months. In interpreting these findings and those in the very interesting case reported by Clover (1958), it is necessary to remember that it is not known whether every untreated case of lymphatic leukosis in dogs necessarily runs a fatal course.

(b) Cat

Apart from the 20 probable cases of lymphatic leukosis, the series of 65 tumours in this system included 31 cases of sarcoma of the mesenteric lymph node, 5 of reticulosarcoma of the spleen, and 9 of lymphosarcoma of the anterior mediastinum: as already mentioned, this latter lesion may occur in cats under 1 year of age.

Lymphosarcoma is clearly an important tumour of cats, and reference should be made by the clinician to the very useful papers by Nielsen and Holzworth (1953), Holzworth and Nielsen (1955) and Holzworth (1958).

7. Tumours of the Urinary System

(a) Dog

TABLE XII

SHOWING CLASSIFICATION OF 52 TUMOURS OF THE URINARY SYSTEM OF DOGS

Site	Type		Number
Kidney			14
	Carcinoma	9	
	Adenoma	2	
	Embryonal nephroma	1	
	Leiomyosarcoma	1	
	Lymphosarcoma	1	
Urinary bladder			36
Benign		12	
	Papilloma	4	
	Fibroma	4	
	Fibroleiomyoma	3	
	Leiomyoma	1	
Malignant		24	
	Carcinoma	21	
	Sarcoma	3	
Urethra			2
	Adenoma	2	
			52

The particularly interesting tumour of this series (Table XII) is carcinoma of the urinary bladder (see Kast, 1956, 1957b, for accounts of renal and urinary tract tumours). There were 21 examples of bladder cancer in dogs. The symptoms were of haematuria. The lesions were often papilliform and sometimes multiple; local infiltration may occur through all coats of the bladder but metastasis was present in only one case. The prospects for operation do not appear to be good.

(b) Cat

There was one possible carcinoma of the urinary bladder. The significant urinary system lesion of cats is the bilateral sarcoma of the kidneys, which is generally a lymphosarcoma and presumably of haematogenous origin.

8. Tumours of the Endocrine System

(a) Dog

The 49 tumours include 40 of the thyroid gland, 7 of the adrenal and 2 of the pituitary. There were no tumours of the parathyroid gland, although Krook (1957) reports parathyroid adenomas in 3 dogs—2 in the normal parathyroid position and 1 at the heart base—and Lamouroux, Lebeau and Sanson (1955) described a tumour in the left thyroid region of an 8½-year-old spaniel which Drieux considered to be a parathyroid tumour.

The thyroid tumours were not easily classifiable as adenomas or carcinomas, but at least 4 of the 12 suspected carcinomas showed metastasis. (Reference should be made to the excellent papers by Clark and Meier, 1958, and Meier and Clark, 1958, for an account of clinical and pathological aspects of thyroid disease in dogs.)

The adrenal tumours included one malignant tumour and one probable ganglioneuroma. No phaeochromocytoma was recognised (see Head and West, 1958, for pathological and pharmacological aspects of a tumour of the canine adrenal medulla).

The two pituitary tumours were probably chromophobe adenomas: one was associated, in a bitch, with bilateral adrenal and ovarian tumours, and multiple mammary tumours.

(b) Cat

There were only two, or possibly three, tumours of endocrine glands: a pituitary chromophobe adenoma, a thyroid carcinoma (c.f. Holzworth, Husted and Wind, 1955) and a possible parathyroid adenoma.

9. Tumours of the Respiratory System

(a) Dog

Tumours of the respiratory system are not common in dogs and in our series those that did occur chiefly affected the nasal passages (see Noack, 1956/57) and 11 only affected the lungs: these comprised 1 connective tissue tumour (possibly a fibroma) and 10 epithelial tumours. Of these 10, 3 were lesions of pulmonary adenomatosis, and the other 7 were probably primary carcinomas (however, only 2 were found in animals autopsied by the writer). Two of the affected dogs had hypertrophic pulmonary osteoarthropathy.

The 31 tumours of the nasal passages included 14 carcinomas. These tumours were often accompanied by unilateral nasal discharges or haemorrhage.

(b) Cat

The 11 malignant tumours of the respiratory system included 3 sarcomas and 2 carcinomas of the nose, a lymphosarcoma and 2 squamous-cell carcinomas of the larynx, and 3 possible primary mucoid bronchial adenocarcinomas (see Stünzi, 1958, for an account of lung cancer in cats).

10. Tumours of the Eye

(Saunders and Barron (1958) have published an excellent account of primary pigmented intra-ocular tumours in animals.)

(a) Dog

The 15 tumours of the eye and adnexa included 5 intra-ocular melanomas, 2 ciliary body adenomas (c.f. Veenendaal, 1958), an intra-ocular tumour which was probably a glioma, an intra-ocular epithelial tumour of unknown type, 5 conjunctival tumours (3 papillomas, 1 sarcoma and 1 melanoma) and a Harderian adenoma.

(b) Cat

Three malignant tumours in the eyes of cats were probably of primary origin—an anaplastic carcinoma of the ciliary body, an anaplastic epithelial tumour and a spindle-cell growth, probably of nervous type. (The involvement of the ciliary body in generalised lymphosarcoma in cats is to be remembered.)

11. Tumours of the Central Nervous System

There were in this series very few examples of tumours of the C.N.S. in dogs and none was seen in cats. Apart from 3 examples of neurofibroma of the facial part of the fifth nerve (see St. Clair and Safanie, 1957) there were 5 canine intracranial tumours: 2 gliomas, 1 astrocytoma, a carcinoma of the choroid plexus of the fourth ventricle, and a malignant meningioma invading the cerebellum. (Stünzi and Perlstein, 1958, have recently given an account of brain tumours in dogs and cats.)

12. Miscellaneous Tumours

Special mention may be made of a "heart-base tumour" in a 5-year-old Spaniel bitch. The animal had been ill for about a week. The symptoms were rather indefinite, the bitch being listless and showing pain on moving. It was particularly reluctant to move the left leg forward. The heart rate was 180. Oedema of the thoracic and abdominal wall developed and the animal was destroyed. An extra-pericardial tumour, 6 cm. long, oval in shape, firm in consistency, brown in colour, faintly lobulated by fibrous tissue, lay on the left side over the right auricle, below and lateral to the trachea. In section, it shows medium-sized cells, which often have a vaguely epithelial appearance, with large oval nuclei, and faintly granular cytoplasm.

Tumours of the heart base are thought to be derived from ectopic thyroid or parathyroid tissue, or from the aortic bodies: this present specimen appears to be of the latter type (see Stünzi, 1952; Jubb and Kennedy, 1957; Kast, 1958; Scotti, 1958).

Discussion

The study of tumours in animals is an extremely fascinating one, but this does not entirely explain why it is being carried on with increasing intensity in many parts of the world by more and more veterinary workers. There are, in the case of tumours of dogs and cats, probably two main reasons for this growing interest—one, a practical one; the other, a recognition of the fact that such a study presents the possibility of clarifying in some significant way certain aspects of the general problem of cancer (Cotchin, 1958b).

(i) From the practical aspect, a careful study by histopathological and other methods of large numbers of tumour cases of different kinds will help to define the range of their biological behaviour, so that not only will soundly-based diagnosis and prognosis be available to the clinician, but information will accumulate which will form a valid key to the interpretation of therapeutic trials, such as those of Mitchell and Silver (1957), P. J. Dalton, Gordon Knight (to speak of England alone) with X-ray therapy, and the extraordinarily interesting work of Livingston (1958) in California in causing regression of certain spontaneous tumours of dogs and cats by long-continued injections of what at first sight would seem a most unpromising material—a filtrate prepared from human placenta undergoing unbuffered non-specific bacterial breakdown at a certain pressure for a prolonged period.

To make this practical aspect of the work really valuable, close collaboration between clinician and pathologist is, of course, essential.

(ii) From the more general aspect, observations such as those reported here present a challenge to our understanding which cannot be ignored: this is that we should try to account for the differences in the incidence and biological behaviour of tumours in different species, for, if we could fully explain these differences, we should have advanced a long way in our knowledge of cancer.

If we look at the material surveyed in the previous pages and take into account what is known about tumours in humans (although even they show remarkable variations according to country, race and so on) we see that some tumours seem to be of importance in all three species—human, canine and feline—others are important in two species, and some are of importance only in one species.

Taking the various body systems in turn:

In the *cutaneous* system, basal-cell and glandular tumours, and squamous-cell carcinomas are significant in all three species. Malignant melanomas are of importance in dog and man, but not in cats. Mast-cell tumours are of importance only in dogs, although they do occur in cats. Ceruminous gland tumours are of significance in cats. Hepatoid adenomas can occur only in dogs.

In the *female genital* system, carcinoma of the uterus and cervix is important in women but must be very rare in dogs and cats. Ovarian tumours are of some importance in women and bitches, but are rare in cats. Mammary adenocarcinoma is important in all three species, but mucoid, chondroid and bony complex tumours, both benign and malignant, are of importance only in dogs.

In the *alimentary* system, cancer of the stomach is of importance only in man, but cancer of the tongue and of the oesophagus are also important in cats. Tonsil cancer is of particular importance in dogs, but is seen to a lesser extent also in cats. Lymphosarcoma of the intestine is important in dogs and cats, while large intestine carcinoma is of great significance only in man.

In the *male genital* system, testis tumours are common in dogs and two of the types which occur—Sertoli-cell tumours (especially) and interstitial-cell tumours—are very much more common in dogs than in man. Prostatic carcinoma is of importance only in man. (The transmissible venereal tumour is, of course, confined to dogs.)

In the *skeletal* system, sarcomas are important in all three species, but the range of type is apparently much narrower in dogs and cats.

In the *lymphatic and haemopoietic* system, lymphosarcoma (including leukosis) is of particular importance in cats and dogs, while myeloid leukosis is of importance only in man.

In the *urinary* system, bladder tumours are of some importance in man and dogs but rare in cats.

In the *respiratory* system, lung cancer is of importance only in man.

Tumours of the *central nervous* system are apparently rarer in dogs and cats than in man.

These differences can be supplemented by others, as listed by Innes (1958) in his masterly chapter on malignant diseases of domestic animals in volume 3 of Butterworth's *Cancer*. It is, of course, necessary to remember that the apparent distribution of tumours amongst the various body systems in surveys such as this will to some extent depend on the source of the material, as operation specimens will tend to be those that occur largely where they are either easily visible to the owner, or produce striking symptoms, or are easily detected on clinical examination (and are, of course, operable). Specimens collected at autopsy will include tumours that may have caused no clinical signs at all, as well as inoperable or metastasised tumours.

However, while we do not at present know how to account for these differences in the important tumours of the different species, it would seem that they must ultimately be based on the fact that dogs are dogs, cats are cats and men are men, with all that that implies in the way of differences in evolution, genotype, phenotype, environment, diet, physiological activities, and so on. If we look at the important kinds of tumour in dogs and cats that have been referred to in the first part of this paper, we find that they present varying aspects that are of special interest, particularly in relation to causation and to histology.

In the case of skin tumours, both aetiological and histological aspects are of interest. The hepatoid tumours, for example, are most commonly seen in ageing male dogs, and it is very probable that an endocrine disturbance is significant in their causation. Many of the lesions of this kind can in fact be classed as hyperplastic rather than neoplastic and in view of the alleviation, albeit temporary, that may result from stilboestrol treatment (Smythe 1945, 1946), these lesions may perhaps be looked on as hormone-dependent adenomas. The ageing male dog is prone to at least two other hyperplastic conditions (enlargement of prostate gland, nodular hyperplasia of interstitial cells of the testis) that are probably signs of endocrine disturbance, and all three lesions may be due to excessive androgen action. Other glandular tumours of the skin of dogs also appear to be more common in males; apart from this, they might provide very useful subjects for studies of histogenesis and for comparison with the lesions of basal-cell and glandular types in man.

The interest of the mast-cell tumours of the skin of dogs, which have been well described and illustrated in recent papers, including those of Larsson (1956, 1957), Head (1958), Nielsen and Cole (1958) and Stünzi (1955), lies in their cell of origin (see Baglioni, 1954; Riley, 1959), their rarity in man, their high incidence in Boxers and their enzyme content (histamine and heparin). The new book by Riley (1959) gives a fascinating account of mast cells and shows in passing how the elucidation of some of the problems of these most interesting cells was helped by studies of dog tumours, in which our veterinary colleague, Mr. K. W. Head, took an important part. The cause of these lesions is not known—some dispute their truly neoplastic nature, at any rate in a proportion of cases, and it is of interest that Lombard and Moloney (1959) report not only successful homologous transplantation of a spontaneous mast-cell tumour through seven generations of 1- to 30-day-old puppies but also successful transmission by a filtrate from a second-generation transplant.

The interest of melanomas of the skin of dogs lies in their breed incidence (being chiefly seen in heavily-pigmented breeds), their sites of origin and their histological structure (they often resemble the "blue naevus" of man rather than the true naevus). Radiosensitivity has been noted for one digital melanoma and this aspect should be studied further (as should the X-ray treatment of canine skin tumours in general —see Riser, 1955).

Referring to the skin adnexal tumours of cats, the ceruminous gland tumours are of interest: whether they are related to the quite common hyperplasia of ceruminous glands, and thus perhaps to otitis, needs study.

As regards the mammary tumours of the bitch, it is possible to refer to only two significant features.

(*i*) *Their Aetiology*. Mammary neoplasms are not known to develop in bitches that are spayed before sexual maturity, although they are occasionally found in bitches spayed when adult (as for pyometra), even one or two years before the tumour is noted. Further, they do not seem to be prone to occur in bitches that breed regularly and it is generally accepted that they are commonly seen in animals that have bred infrequently, if at all, and also (although the point needs confirmation) in bitches that have shown abnormalities of the sexual cycle. Perhaps repeated pseudo-pregnancies are the basic cause of these tumours: the view might even be expressed that pseudo-pregnancy is an abnormal physiological state. Since differences in sexual and breeding cycles might be significant in relation to the common occurrence of these mammary tumours, my colleague, Mr. Frost, is making an ex-

tensive survey of the histories of bitches with and without tumours, and we are also making a closer study of the pituitary glands, adrenals and ovaries of bitches coming to autopsy to see if the endocrine status of affected and non-affected bitches can be more clearly defined.

(*ii*) *Their Histogenesis.* Most of the benign mammary tumours of bitches are partly or wholly mixed in type, showing often a variable formation of mucoid, chondroid and bony tissue. The histogenesis of some of this complex tissue is most probably by way of proliferation of myoepithelial cells, which become embedded in a mucoid material, which is then in turn changed into cartilage and later, by endochondral ossification, into bone. Some of the bone and cartilage in these tumours is also formed by metaplasia of intralobular connective tissue, possibly under the inductive influence of the mammary epithelium.

The probable myoepithelial involvement in benign lesions is a striking enough feature of bitch mammary tumours but what is even more striking is that many of the malignant tumours of the mammary gland are not ordinary carcinomas, derived from duct or acinar lumenal epithelium, but are often sarcomas, frequently mucoid sarcomas, or chondrosarcomas, or, not rarely, osteosarcomas, which may themselves possibly be of myoepithelial origin.

Finally, more striking still is the occurrence of what seem to be truly mixed malignant tumours, that is carcinosarcomas, apparently derived from both lumenal epithelium and myoepithelium, each component apparently being malignant and capable of metastasising.

In the *alimentary* system, the tumours of particular interest are the tongue and oesophageal carcinomas of cats and the tonsil carcinomas of dogs.

It would seem quite possible that the tumours of the upper alimentary tract of the cat could be due to something that the cat eats, drinks, or otherwise swallows. Milk and fish might come under review, but perhaps the most likely cause might be the swallowing of some carcinogenic factor contaminating the fur of the town cat. However, not all these cancers were in London cats—we have examples of tongue carcinoma in a cat in Sussex, of tonsil carcinoma in a cat in Wiltshire and of oesophageal carcinoma in a cat in Essex. The figures of incidence in Glasgow, Edinburgh and London cats might be worth comparing.

Again, it would be of interest to know the general incidence of tonsillar carcinoma in dogs in Britain: while this type of cancer in rarely seen in Switzerland (Stünzi and Rusterholz, 1958) and is apparently rare in Austria (Eikmeier and Sandersleben, 1955) it is certainly of some importance in the dogs seen here.

As regards *testis* tumours, their interest in the dog lies in the frequent occurrence of Sertoli-cell tumours (which are very rare in man), and the fairly common signs of endocrine upset, as evidenced by alopecia and feminisation, not always definitely related to Sertoli-cell tumours. The relationship of tumour development, particularly of Sertoli-cell tumour development, to ectopia testis needs investigating further.

The interest of *bone* sarcomas in dogs lies in their common occurrence in the long limb bones, where they arise (except in the case of the tibia) almost invariably in the region of the longer of the two epiphyseal lines—upper humerus, lower radius, lower femur —in their particularly important incidence in large dogs (Great Danes, for example) and in the relatively high incidence of rib sarcomas—these tending to occur in young dogs. The significance of this special breed and site incidence of limb bone tumours in dogs is not clear. Schulz (1958; Brass and Schulz, 1958) has suggested a possible relationship to previous traumatic injury in the development of bone sarcoma in dogs, perhaps complicated by disturbed healing processes. Incidentally, Banks and Bridges (1956) have reported a unique case of multiple cartilaginous exotoses in a 9-year-old dog, with chondrosarcoma development on the spinous process of the third thoracic vertebra.

The common incidence of *lymphosarcoma* in dogs and cats would seem to offer the opportunity of both aetiological and chemotherapeutic studies.

In the *urinary* system, it is of interest that of the 21 specimens of carcinoma of the urinary bladder of dogs, 15 have been examined since 1954 and no less than 8 such tumours were examined last year.

In this recent series of 15 bladder cancers, 9 were in bitches and 5 of the affected animals were Scottish Terriers. The question might be posed: Have dogs in recent years been more exposed than previously to carcinogenic substances that might act like β- naphthylamine?

In the *respiratory* system, the infrequency of lung cancers in dogs in London might be due to the fact that they do not live long enough for any carcinogenic action by the polluted air they breathe (cats seem to have a better nasal filtering action, as their lungs are not so anthracotic as those of dogs). There is at present no indication of an increase in the incidence of primary lung cancer in dogs in London, as was suggested for dogs examined in Utrecht (ten Thije and Ressang, 1956; Ressang, 1956).

Other questions will no doubt suggest themselves to those perusing the surveys given above.

There is perhaps one major field in which the study of animal tumours could be developed—that of therapy. In this respect, it is important to remember that the welfare of the animal affected with a spontaneous tumour must always be a prime consideration. For this reason, the study of different possible lines of treatment of tumours, whether by X-rays or by chemotherapy or otherwise, might be encouraged, for positive findings, while they would probably lead to corresponding benefits to mankind (bearing in mind the care with which results in animals can be extrapolated to man), would certainly be of benefit to affected dogs and cats.

Other developments in the study of spontaneous tumours in animals that would be welcomed would include the preparation of an atlas of animal tumours to meet the need for consistent reporting and for stimulation of comparative studies; the development of animal tumour registries in different parts of the world, to collect and collate material and help in epidemiological and other studies; the encouragement of those already working on animal tumours and of others who might become interested.

The development of comparative oncological

studies must be a two-way process: on the one hand, the veterinary worker will be able to bring forward problems such as those touched on earlier in this discussion, while on the other hand the worker concerned with cancer studies in man or in experimental animals would surely do well to bear in mind the occurrence of spontaneous tumours in domestic animals in planning, executing or evaluating his own studies, or in constructing theories of the nature of cancer.

It has often been stated that more use should be made of the material provided by the common occurrence of spontaneous tumours in domesticated animals. It should be clear from what has already been said that it is not the purpose of this paper to advocate the use of healthy dogs and cats in experimental cancer research: what is being urged is that, faced with the fact that tumours do frequently occur in these animals, we should make as careful a study as possible of various aspects of these tumours in the hope not only of throwing some light on the general problems of cancer, but also of benefiting the affected species themselves. It will therefore be of interest to see how the observations reported here appear to someone who has long been engaged in what might be called "orthodox" fields of cancer research, and to learn how he would consider that comparative oncology might best be developed.

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References

BAGLIONI, T. (1954). *Att. Soc. Ital. Sci. vet.*, **8**, 145-222.
BAKER, E. (1956). *Jour. Amer. vet. med. Assn.*, **129**, 322-4.
BANKS, W. C. (1955). Ibid., **127**, 446.
BANKS, W. C., and BRIDGES, C. H. (1956). Ibid., **129**, 131-5.
BERG, O. A. (1958). *Acta endocrinologica*, **27**, 140-54.
BLOOM, G., LARSSON, B., and ÄBERG, B. (1958). *Zbl. Vet. Med.*, **5**, 443-58.
BRASS, W., and SCHULZ, L. C. (1958). *Dtsch. tierärztl Wschr.*, **65**, 594-9.
BRODEY, R. S., and MARTIN, J. E. (1958). *Jour. Amer. vet. med. Assn.*, **133**, 249-57.
BRODEY, R. S., MCGRATH, J. T., and REYNOLDS, H. (1959). Ibid, **134**, 53-71.
CELLO, R. M., and KENNEDY, P. C. (1957). *Cornell Vet.*, **47**, 538-57.
CLARK, S. T., and MEIER, H. (1958). *Zbl. Vet. Med.*, **5**, 17-32.
CLOVER, R. H. (1958). *Vet. Rec.*, **70**, 742-7.
COTCHIN, E. (1947). *Proc. Roy. Soc. Med.*, **40**, 636-7; (1951) *Vet. Rec.*, **63**, 67-72; (1954a) Ibid., **66**, 879-94; (1954b) *Brit. Vet. J.*, **110**, 218-30, 274-86; (1955) *J. comp. Path.*, **65**, 115-29; (1956) Neoplasms of the domesticated mammals; Review Series No. 4, Commonwealth Agricultural Bureaux; (1957) *Vet. Rec.*, **69**, 425-34; (1958a) *J. comp. Path.*, **68**, 1-22; (1958b) *Vet. Rec.*, **70**, 752-5.
EIKMEIER, H., and SANDERSLEBEN, J. v. (1955). *Wien. Tierärztl. Mschr.*, **42**, 886-93.
GANS, J. H. (1958). *Cornell vet.*, 48, 372-7.
GRANT, C. A. (1957). *Acta. path. microbiol. scand.*, **40**, 197-208.
GROTH, W. (1955). *Z. Krebsforsch.* **60**, 361-72.
HATAYA, M., SHIRASU, Y., and TAKEUCHI, A. (1958). *Gann*, **49**, 307-18.
HEAD, K. W. (1958). *Brit. J. Dermatol.*, **70**, 389-408.
——, and WEST, G. B. (1958). *J. comp. Path.*, **68**, 167-72.
HOLZWORTH, J. (1958). *Jour. Amer. vet. med. Assn.*, **132**, 124-30.
HOLZWORTH, J., HUSTED, P., and WIND, A. (1955). *Cornell Vet.*, **45**, 487-96.
——, and NIELSEN, S. W. (1955). *Jour. Amer. vet. med. Assn.*, **126**, 26-36.
INNES, J. R. M. (1958). *Cancer*, Vol. 3, pp. 73-115. London: Butterworths.
IRFAN, M. (1958). Studies on the peripheral blood picture of the dog and cat in health and disease. Ph.D.Thesis, London.
JUBB, K. V., and KENNEDY, P. C. (1957). *Cancer*, **10**, 89-99.
KAST, A. (1956). *Mh. Vet. Med.*, **11**, 483-90; (1957a) Ibid, **12**, 212-6; (1957b) Ibid, **12**, 154-6; (1958) *Zbl. Vet. Med.*, **5**, 459-74.
KROOK, L. (1957). *Acta path. microbiol. scand.*, **41**, *Suppl.* 122, 88 pp.
LAMOUROUX, LEBEAU and SANSON (1955). *Bull Acad. vét. Fr.*, **28**, 25-9.
LARSSON, B. (1956). *Nord. Vet. Med.*, **8**, 130-9; (1957) Ibid., **9**, 241-56.
LIESEGANG, N. (1958). *Mh. Vet. Med.*, **13**, 552-3.
LIVINGSTON, W. S. (1958). *J. Nat. Cancer Inst.*, **20**, 245-307.
LOMBARD, L. S., and MOLONEY, J. B. (1959). *Fed. Proc.*, **18** (1), No. 1931, p. 490.
MCCLELLAND, R. B. (1957). *Cornell Vet.*, **47**, 533-8.
MEIER, H. (1956). *N. Amer. Vet.*, **37**, 979-81; (1957a) *Zbl. Vet. Med.*, **4**, 633-88; (1957b) *Cornell Vet.*, **47**, 220-6.
——, and CLARK, S. T. (1958). *Zbl. Vet. Med.*, **5**, 120-8.
MITCHELL, J. S., and SILVER, I. A. (1957). *Vet. Rec.*, **69**, 1376-85.
MULLIGAN, R. M. (1955). *Amer. J. Path.*, **31**, 773-89.
NIELSEN, S. W., and COLE, C. R. (1958). *Amer. Jour. vet. Res.*, **19**, 417-32.
——, and HOLZWORTH, J. (1953). *Jour. Amer. vet. med. Assn.*, **122**, 189-97.
NOACK, P. (1956/57). *Wiss. Z. Humboldt-Univ.*, **6**, 293-314, 373-91.
PATTERSON, D. F., and MEIER, H. (1955). *Jour. Amer. vet. med. Assn.*, **127**, 495-8.
RESSANG, A. A. (1956). Primary lung carcinoma in the dog. Bogor: State University of Indonesia, 450-84.
RILEY, J. F. (1959). The mast cells. Edinburgh and London: E. & S. Livingstone.
RISER, W. H. (1955). *Mich. St. Coll. Vet.*, **15**, 199-204.
——, MARCUS, J. F., GUIBOR, E. C., and OLDT, C. C. (1959). *Jour. Amer. vet. med. Assn.*, **134**, 27-8.
SANDERSLEBEN, J. v. (1956). *Mh. Tierheilk*, **8**, 1-8.
SAUNDERS, L. Z., and BARRON, C. N. (1958). *Cancer Res.*, **18**, 234-45.
SCHULZ, L. C. (1958). *Z. Krebsforsch.*, **62**, 278-90.
SCOTTI, T. M. (1958). *Jour. Amer. vet. med. Assn.*, **132**, 413-9.
SMYTHE, R. H. (1945). *Vet. Rec.*, **57**, 115; (1946) Ibid., **58**, 75.
ST. CLAIR, L. E., and SAFANIE, A. H. (1957. *Jour. Amer. vet. med. Assn.*, **131**, 188-91.
STÜNZI, H. (1952). *Schweiz. Ztschr. Allg. Path. Bakt.*, **15**, 631-7; (1955) Ibid., **18**, 1271-7; (1958) *Zbl. Vet. Med.*, **5**, 665-74.
——, and PERLSTEIN, Z. (1958). *Schweiz. Arch. Tierheilk.*, **100**, 139-57.
——, and RUSTERHOLZ, P. (1958). Ibid., **100**, 271-7.
——, and SUTER, P. (1958). *Mh. Vet. Med.*, **13**, 251-6.
TEN THIJE, J. H., and RESSANG, A. A. (1956). *Dtsch. tierärztl. Wschr.*, **63**, 17-8.
ÜBERREITER, O. (1955). *Wien. tierärztl. Mschr.*, **42**, 893-904.
VEENENDAAL, H. (1958). *Tijdschr. Diergeneesk.*, **83**, 329-44.
YOST, D. H., and JONES, T. C. (1958). *Amer. Jour. vet. Res.*, **19**, 159-63.

THE SPEAKER'S INTRODUCTION

The Chairman, Mr. D. G. Lewis, introduced Dr. Cotchin, whom, he said, most of those present knew very well either from teaching days or from the help that he had given them in practice.

Dr. E. Cotchin, in presenting his paper, said that from several years' experience, the results of which were summarised in the paper, a fairly clear picture was emerging of what kinds of neoplasms were of importance in dogs and cats in what might be called the diagnostic catchment area of the London College. It was quite possible that this picture was badly distorted by known and hidden biases in the collection

of material, but there were two main factors which would prevent it from being universally applicable—on the one hand, place; on the other, time.

For example, the possibilities of geographical variation in the incidence of tumours in animals, such as were known to occur in man, were being increasingly brought into consideration—the importance of tonsil cancer in these dogs was a case in point. These geographical variations might occur even in such a small area as this country itself, and it would be of interest to know whether any members present had noted any particularly high incidence of any sort of tumour in their practice. Such variations might point to important aetiological factors, and they needed to know a lot more about the epidemiology of animal tumours.

On the subject of variations with time, this was a difficult problem on which to form definite opinions. It did seem pretty certain, however, that more cases of mast-cell tumour would come to notice with the increased popularity of Boxers, and in the paper too he had noted that they had recently seen an increased number of bladder cancers in dogs. Where only small numbers of tumours were concerned, it was impossible to be certain whether increases in incidence were real, but even indications of possible trends in incidence might be significant.

At one time it had been thought that the main reason why certain tumours that were common in man were not often seen in animals was that man lived longer. However, they saw that in the two domestic species dealt with, which by and large tended to live out much if not all of their natural life span, the tumour spectrum differed as between the two species, and as contrasted with man, although all three species lived in much the same environment. Perhaps not enough attention had been given to the idea that the important tumours of man were largely specific to humans.

For his own part, he would like to learn from this meeting, firstly, something as to the importance of tumours in animals as a problem in clinical practice, and secondly, any views that members might have on the following points: (1) Were there any special tumours that were important, either by their occurrence or by their non-occurrence, in particular practices? (2) Had any member any particular therapeutic measures he had found of value in certain tumour cases? (3) Had any member formed any views of an original, even unorthodox, kind, on the problem of cancer as a result of his experience of animal tumours?

He wished to show a few lantern slides to illustrate the commoner tumours of dogs and cats, and also to include a few pictures of human cancers. Before showing the slides, he wished to express his sincere appreciation of the fact that Professor Haddow had found time amongst his many activities to come along today to give them the benefit of his views, and to do him the great honour of opening the discussion on the paper.

The Opener

The Chairman said that it was a great honour for the Association to have Professor Haddow open the discussion on the paper. Professor Haddow was one of the outstanding figures in cancer research in the world today.

Professor A. Haddow, in opening the discussion, said that his first and very pleasant duty was to thank the Association for the invitation to do so, which he had been very happy to accept. It gave him the opportunity to acknowledge the debt that cancer research owed to the veterinary profession. It also afforded him a good deal of personal pleasure to comment upon Dr. Cotchin's paper.

He often thought that when the history of cancer research came to be written, perhaps the main part of the contribution would flow from animal studies, and from the knowledge gained from direct observation of the disease in animals, domestic and others.

He had himself benefited in his working life from many contacts with the veterinary world. In Edinburgh, before he had left it 25 years ago or more, he had had many fruitful associations with the Dick, and on coming to London and ever since he had had a great deal of help from the Royal Veterinary College, particularly from Professor Wright in the old days, from Mr. Gordon Knight, and of course from Dr. Cotchin himself. Nevertheless, he believed that there was a great deal more to do as between the veterinary side and his own side, as he hoped to show arising from Dr. Cotchin's remarks.

The present paper brought up to date Dr. Cotchin's earlier series. He did not wish to flatter Dr. Cotchin, but he thought that, taken together, all these papers represented a most careful, studious and, he ventured to say, would prove to be even a classical account which had been very greatly appreciated and valued already, especially by reason of the comparative interest as between the veterinary and the human fields.

Dr. Cotchin had carried out a survey of upwards of 4,000 tumours in dogs and upwards of 500 in cats, in the Department of Pathology of the Royal Veterinary College, over the past 18 years. Dr. Cotchin had described carefully the age incidence and the breed incidence. Certain examples of breed variation were particularly interesting, especially the liability of the Boxer to mast-cell tumours and the liability of the Great Dane to sarcoma of the bone. Then there was the sex incidence, with many fascinating observations and the preponderance of males in circumanal adenoma of the dog, and probably carcinoma of the upper alimentary tract of the cat. Lastly, there was a good and full account of the systems of origin of all those different neoplasms.

He was very struck by the rarity of carcinoma of the lung, stomach, large intestine or uterus in dogs and cats, in contrast with the importance of these tumours in human beings. On the other hand, many tumours in dogs and cats were rare in humans—e.g., mast-cell tumours of the skin in dogs, mixed mammary tumours of the bitch, and Sertoli-cell tumours of the testis of dogs. Dr. Cotchin had drawn a fascinating comparison between the human, canine and feline species.

Some tumours were prominent in all three, others in two of the species, or only in one—reflecting fundamental variations in the genetic, environmental, nutritional or endocrine background.

In the female genital system, there was the importance of carcinoma of the uterine body and cervix in women, and the rarity in dogs or cats; also, there was the rarity of ovarian tumours in cats. Mammary adenocarcinoma was important in all three species, but the mucoid, chondroid and bony complex tumours, both benign and malignant, were of importance only in dogs.

In the alimentary system, cancer of the stomach was important in man, while cancer of the tongue and oesophagus also occurred in the cat. The frequency of tonsillar carcinoma in dogs was also important, as was the observation of lymphosarcoma of the intestine in dogs and cats, with cancer of the large intestine significant only in man.

Dr. Cotchin had put forward various proposals for the advancement of research, such as the preparation of a definitive atlas of animal tumours, and the development of animal tumour registries. Most of them would approve and support ideas of that sort as being useful steps towards the completion of their knowledge.

He had been very interested to read Dr. Cotchin's remark that he would not wish in any way to advocate the use of healthy dogs and cats in experimental cancer research. He (Prof. Haddow) heartily approved and endorsed that point of view. For many years, and much more as time went on, he had been very thankful in his own work that it had not been necessary to involve the dog or the cat. He felt that they must treat every animal with responsibility. In the words of Blake, everything that lived was holy. But from time immemorial the dog and the cat had had the special place in the affection of man, and seemed to call for a special protection.

There was, however, no doubt, as Dr. Cotchin suggested, that the study of spontaneous tumours in dogs and cats should be greatly developed—by histological, histogenetic and other analyses. He made a plea for a much more systematic cytological studies of the chromosome complex, which was greatly neglected in both species.

Dr. Cotchin also suggested that these cases should be used in therapeutic studies. He quoted references to the use of

chlorambucil—which had been developed on the basis of experimental chemotherapy studies in the rat and then applied directly to treatment in man—and also to the use of cortisone in the therapy of the lymphatic leukoses in dogs. No doubt there was a great future for the chemotherapeutic treatment of animal tumours, and a great opportunity for collaboration as this work developed over the years.

In another field, he had the impression that much more could be done to elucidate the endocrine background of many spontaneous animal tumours.

Dr. Cotchin had pointed out that mammary tumours in cats were usually seen in entire females but were not restricted to them, and that mammary neoplasms were not known in bitches spayed before sexual maturity, although occasionally in bitches spayed when adult. They were not prone to occur in bitches that bred regularly, but rather in animals that had bred infrequently, if at all, and also perhaps in bitches that had shown abnormalities of the sexual cycle. All these observations and findings were relevant to experience in other species, especially, of course, in the mouse. Since differences in sexual and breeding cycles might be significant in causation, studies were being made by Frost of the pituitaries, adrenals and ovaries of affected and non-affected animals.

There were many other ways in which the veterinarian could help in cancer research. He was sure that each year there must be a great deal of potentially important material passing through veterinarians' hands which for one reason or another was lost to the research workers. Many of the reasons were natural and understandable, but he was sure that really valuable material could be picked up much more frequently than it was at present.

From Dr. Cotchin's review, he got the impression, also, that many of the tumour types rather specific to the species or characteristic in them were more frequent of occurrence than in man or were different from those occurring in man, and this presented veterinarians with rather special opportunities in the course of their clinical observation.

To quote one example which had intrigued him two years ago, Miss Joshua had made an observation about which she had consulted him at the time, namely, that the removal of a nose in a dog with lymphatic leukaemia was followed by a very remarkable and generalised regression of the lesions elsewhere, and that was an observation which he understood was not unique in cases of the kind which had been observed. They had tried to duplicate that experience in animal tumours in the mouse and the rat, but with completely negative results. He quoted that observation of Miss Joshua's as being of the kind that could be of immense importance, and for the making of which he thought that veterinarians might have very special opportunities.

Why he was worried at the loss of potentially important material was that where observations had been made, especially experimentally, in the field of animal tumours, they had often been proved to be literally key observations which had affected the whole of the subject. Perhaps the greatest of all was that of Rous in 1910, which had been a fundamental observation. An immense amount of work still proceeded on it, but there was no question whatever that when more was known of the physical and chemical make-up of this remarkably specific particle, there would be profound effects upon the whole development of cancer research.

Another well-known example was the discovery of the Bittner agent in mammary cancer in mice in 1933, which had come originally from a keen observation made by a laboratory technician.

As they knew, in the past 3 to 5 years amazing strides had been made in the study of the so-called polyoma viruses in mice. These were agents which were injected into day-old or very young animals, and within a few months induced not only euphemias in many cases, but also a great range of some 20 or 30 tumour types which were again transmissible by the same means and, contrary to experience so far, were transmissible not only in the same species but also in others, as, for example, hamsters.

As Dr. Cotchin had indicated, there was, of course, an increasing interest in tumours of domestic animals. But there was a need for expansion of veterinary research in this field, as doubtless in others; and certainly there was a need for ever greater co-operation with the medical side.

This, he supposed, raised matters of research policy. Such a desirable expansion need not, he thought, be expensive. A good deal could be achieved by more active co-operation in cancer research; but nevertheless he did not think there was any doubt that more funds and facilities were required, and the means should be found.

In contributing towards these ideals and the advancement of knowledge, few had done more than Dr. Cotchin. In closing, he wished to thank Dr. Cotchin again, and the Association, for permitting him the privilege of opening the discussion on what he was sure was a paper of permanent and serious interest and value.

The General Discussion

Mr. K. W. Head (Edinburgh): "I have found this paper of great interest because our collection of tumours in the Pathology Department of the Royal (Dick) School of Veterinary Studies, Edinburgh, extends over almost the same time span as that of Dr. Cotchin (1st August, 1940, to 31st July, 1957, as compared with 1940 to 1958). It was obtained from similar sources and mainly, but not exclusively, from South-east Scotland. During this period we recorded 4,555 tumours of dogs and 413 tumours of cats, comparable numbers to the London figures of 4,187 and 571 respectively. I have made a comparison between the site distribution figures of the London and Edinburgh material, checking any obvious differences by the Chi-square test and find a large measure of agreement between the tumour patterns in these two centres.

TABLE I

COMPARISON OF DISTRIBUTION OF CLASSIFIED SKIN TUMOURS IN DOGS

Type	Number of cases			Significance
	Cotchin	Head		
		Expected	Observed	
Papilloma	18	24·1	150	High
Fibroma	130	173·9	214	Definite
Basal-cell and glandular tumours	395	528·5	588	Definite
Mast-cell tumours ..	91	121·8	184	None
Lipoma	52	69·6	116	None
Angioma	43	57·5	69	None
Squamous-cell carcinoma	76	101·7	108	None
Sarcoma	314	420·1	251	Definite
Melanoma	254	339·8	157	Definite
Total all types	1,373	1,837·0	1,837	

"There are, however, significantly more skin tumours in the Edinburgh sample (see Table I), which could be accounted for by a conscious selection on my part, as I have been especially interested in these neoplasms. Using the totals of the number of skin tumours observed at both centres, one can calculate from the number of each type of skin tumour seen by Dr. Cotchin, the number of each tumour type which should be expected in my series. When these expected values are compared with the observed values, certain groups are significantly more numerous in the Edinburgh and, conversely, other tumour types are more numerous in the London figures (see Table I). This may be explained by the postulation that two further factors operate to alter the pattern of samples.

"Firstly, the bulk of my skin tumour material was obtained by examining every tumour (however small) removed by the Department of Surgery, R.(D.)S.V.S., irrespective of whether or not a diagnosis could be made at the time of operation or whether malignancy was suspected. This may account for the large number of papilloma and basal cell and glandular tumours recorded. Secondly, the differences noted in the distribution in fibroma, sarcoma and melanoma may be due to different criteria for classifaction. I will be interested to hear Dr. Cotchin's views on this suggestion.

TABLE II

COMPARISON OF DISTRIBUTION OF BONE TUMOURS IN THE DOG AND CAT

Site	Dog			Cat		
	Cotchin	Head		Cotchin	Head	
		Expected	Observed		Expected	Observed
Humerus	35	15·5	6†	15	4·3	3*
Rib	21	9·3	5*	0	0·0	1*
Femur	17	7·5	4*	10	2·9	2*
Tibia	22	9·7	9*	1	0·3	1*
Radius	20	9·0	11*	3	0·9	1*
Other sites	39	17·0	33‡	16	4·6	5*
Total	154	68·0	68	45	13·0	13

Significance: *None; †Probable; ‡High.

Certainly, these facts re-emphasise Dr. Cotchin's plea for an atlas of Veterinary Tumour Pathology so that workers in different centres may record their findings in such a manner that they can be easily and accurately compared.

"We have seen significantly fewer bone tumours in dogs and cats than Dr. Cotchin. Although the site distribution of these tumours in the cat is comparable in the two series, in the dog there are two obvious differences, that is, the Edinburgh material has fewer bone tumours of the humerus and more at 'other sites'. Ten of these 'other site' tumours occurred in the nose and this may be significant in view of the fact that Dr. Cotchin records 31 tumours in the nasal passages, whereas we only found 16 (epithelial tumours, mainly adenocarcinoma). Would Dr. Cotchin care to express an opinion as to the reason for these differences?

TABLE III

COMPARISON OF DISTRIBUTION OF CARCINOMA OF THE ALIMENTARY TRACT OF THE CAT

Site	Number of cases			Significance
	Cotchin	Head		
		Expected	Observed	
Pancreas	9	5·0	11	Probable
Tongue	18	10·0	13	None
Intestine	11	6·2	8	None
Liver	6	3·3	5	None
Gum, palate and pharynx	17	9·5	9	None
Parotid	2	1·1	1	None
Tonsil	13	7·2	3	None
Oesophagus	21	11·7	4	Probable
Total all sites	97	54·0	54	

"Although there is no significant difference between the total number of carcinoma of the alimentary tract in the cat between the two series, there is an obvious variation in the frequency with which the pancreas and oesophagus are involved. Is this a true geographical difference in distribution of these tumours, or does it perhaps reflect a difference in the population from which we draw our material? I have discussed previously the problems of assessing the normal population from which my tumour material has been obtained [Head 1958(a)]. A 'normal sample cat population' figure can be obtained by adding Dr. Cotchin's data for the tumour-bearing and clinic cats (see Table IV); the Edinburgh 'normal sample cat population' figures were obtained from all cats autopsied or from which biopsies were examined during the period 1st August, 1941 to 31st July, 1953. From Table IV it can be seen that the age distribution graph is comparable (though with a somewhat sharper fall) but that the sex ratio is closer in the Scottish series. Is the variation in the pancreatic and oesophageal tumours due to a variation in sex distribution of the population, or due to some other factor?

TABLE IV

COMPARISON OF THE AGE AND SEX DISTRIBUTION OF "NORMAL SAMPLE POPULATIONS" OF CATS

		Number observed		Percentage distribution	
		Cotchin	Head	Cotchin	Head
Age in years:	0-5	524	258	49·0	60·3
	6-10	328	106	30·7	24·8
	11-15	190	51	17·7	11·9
	over 15	28	13	2·6	3·0
Total		1,070	428	100·0	100·0
Sex: Male (entire and castrated)		774	396	74·8	57·9
Female (entire and spayed)		261	288	25·2	42·1
Total		1,035	684	100·0	100·0

"Finally, with regard to Dr. Cotchin's plea for the increased use of spontaneous cancer cases in dogs and cats in the evaulation of various therapeutic measures. Our experience with mast-cell tumours bears of Dr. Cotchin's statement that, firstly, 'the range of biological behaviour' of each tumour type must be established before the effective therapy can be judged. Thus, mast-cell tumours may change their macroscopic and microscopic features quite dramatically during their natural history [Head 1958 (b)]—a phenomenon which is now appreciated and would make the interpretation of therapeutic trials in this disease extremely difficult.

"I would like to congratulate Dr. Cotchin on the immense amount of work which has gone into the preparation of this paper and tell him that I admire the way in which he has achieved a success that so frequently eludes me, namely, that the figures of his various tables all add up to the correct total given!"

References

HEAD, K. W. 1958. (*a*) *Proc. Book First Ann. Congress Brit. Small-Animal Vet. Assoc.* (52-62).

HEAD, K. W. 1958. (*b*) *Brit. J. Dermatol.* **70,** (389-408).

The Replies

Dr. E. Cotchin, in replying to the discussion, thanked Professor Haddow for the excellent way in which he had opened the discussion. Professor Haddow had made a lot of rather flattering remarks which could be discarded, but the main point that he had made was that there should be an expansion of support for research in this field. He (Dr. Cotchin) entirely supported this, as he hoped others would do.

To reply briefly to some of the other contributors to the discussion, it would have been noted that Mr. Head had examined a good many more tumours than he had done, and he hoped that Mr. Head would find time, or be given time, to put these things on record and publish the results

so that others could have the benefit of seeing them. He thought that in general one might say that difficulties with regard to background population were going to be important in comparing London, Glasgow and Edinburgh animals. His own paper looked very neat and tidy and the figures all added up, as Mr. Head had said, but there was a cloud of difficulties and obscurities to be found within the figures—e.g., how many Great Danes there were in Scotland, and so forth; and that sort of thing was bound to have an influence on the final table. He was certain that difficulties of classification were important, and if it were possible to get the atlas to which he had referred, then one would know that Mr. Head, himself, and others were talking about the same things.

Mr. Collins of Australia had said that he was disturbed to get a report of lymphosarcoma when he thought he was dealing with a case of lymphatic leukosis. He should not have been disturbed, because the tendency nowadays was to consider that there was a malignant tumour of the lymphatic tissue which was called in general lymphosarcoma. Sometimes it affected a local area and appeared as a solitary tumour and in other cases it was rather more generalised. In the case of the intestine of the cat and the dog one often found multiple tumours, and it was thought that perhaps lymphatic leukaemia was the most generalised form of this neoplasm of lymphatic tissue. It might be a matter of chance whether it affected the whole lymphatic system or one particular part.

Mr. Osborne had referred to the term "pseudo-Hodgkin's disease". The only resemblance to Hodgkin's disease was that there was an involvement of the lymphatic tissue, particularly in the neck region. In the old days, the veterinary clinician had called it "pseudo-Hodgkin's disease" and he had also done so in the paper, but he would agree that they might put the term into cold storage for a bit.

On the question of the origin of alimentary tumours in the cat, Mr. Osborne had said that if the tongue tumour was due to licking of contaminated fur perhaps the tumour ought to be on the top of the tongue. He thought that there was the alternative possibility that material licked into the mouth of the cat would be cleaned off the tongue and swallowed, whereas there was the chance of it hanging about a little longer under the tongue. With regard to cancer of the oesophagus, the possibility was that when the cat swallowed the material it passed down the oesophagus and was held up for a fraction longer in front of the heart, so that over the course of many years that particular part of the oesophagus was exposed for, say, 120 per cent. of the time that the rest of the oesophagus was, and that might be just enough to bring it into period necessary for carcinogenesis.

He was sure that Professor Weipers was right in his remarks about mammary cancer. If it were possible to carry out a *post-mortem* examination on every animal with a mammary tumour, he was sure that one would pick up more secondaries.

Miss Brancker had asked about the difference between London dogs and cats and non-London dogs and cats. Much of the material that came in through the clinic was in fact country material, and conversely, some of the material that was sent in by country practitioners was in fact London material. He would say that the majority was London material, but he could not say what the exact proportion was.

With regard to the significance of the castrated male, in humans it was usually said that alimentary cancer of this kind was more often seen in males, and the suggestion was made that this was due to the fact that the male hormone was related in same way to the susceptibility to cancer. With cats, where there were corresponding tumours, it was found that the cats were the castrated ones. He thought they should say that in the case of man with alimentary cancer, the negative fact that he was not a female appeared to predispose him to it, rather than the positive fact that he was a male. So perhaps femaleness protected against alimentary cancer, rather than maleness predisposing to it.

Professor A. Haddow, who also replied, said that Mr. Head had raised a very practical point in relation to studies of chemotherapy. Some years ago they had carried out a controlled study of the behaviour of mammary cancer in the mouse over many hundreds of cases under normal conditions, and they had been astonished to find the extent of variation which was seen and which could very easily be attributed to treatment being undertaken. It was absolutely essential to have that background before such trials were conducted. The same had happened in the case of a study of mammary cancer in man. Spontaneous variations had been much more frequent than one would perhaps realise if a definite study were not made.

Many of the points that had been made in the discussion seemed to show even more clearly than before the need for some of the measures that Dr. Cotchin had proposed. The difficulties in the mater of diagnosis emphasised the need for a pathological atlas or some method which would afford much greater standardisation and rationalisation of tumour nomenclature.

Miss Brancker had also shown very clearly the need for much better statistical precision before one could draw conclusions, and Mr. Head had shown what very wrong conclusions could easily be drawn in the absence of that statistical precision. They needed first of all accurate figures as to the animal population in this country and the age distribution. Here again, Dr. Cotchin's reference to the need for a tumour registry was a suggestion which, if carried out, would take care of regional differences as well as differences between London and other parts of the country. The discussion had shown the need for these improvements and developments, and he felt sure that this meeting would result in some of the progress that Dr. Cotchin and all of them wanted to see.

The Chairman proposed a vote of thanks to Dr. Cotchin and to Professor Haddow.

Progeny Testing of Farm Livestock

BY

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IN this paper no attempt is made to review the very voluminous literature on the subject of progeny testing which has appeared in recent years; the object is, rather, to examine certain aspects of progeny testing in relation to the practice of livestock production and its improvement. The great majority of stockbreeders have utilitarian aims—to condition their stock to supply human needs as efficiently and with as satisfactory an economic return as possible. The role of progeny testing as an aid to the breeding of better stock must therefore be judged in the broad practical context of livestock industries.

Stockbreeders have many methods of selecting from among the present generation of animals those which are regarded as worthy to be parents of the next generation in the sense that their offspring may be likely to show higher or more consistent standards of performance. Some of the methods have proved inaccurate and wasteful in their results; increasing knowledge of genetical principles and of the modes of inheritance of performance characters has provided explanations of mistakes and failures and pointed to ways by which the accuracy of parental selection can be improved. Modern developments have been mainly aimed to make selection methods more precise; recent developments of statistical concepts and techniques have greatly helped. While selection on progeny performance is by no means a new method for stockbreeders to use, the newer techniques have brought out more clearly, on the one hand, the requirements to be met in adequate progeny tests and, on the other hand, some of the limitations of their use and interpretation.

The essential features of the situation are well known and can be briefly restated thus: some characters are simply inherited, some have more complex modes of inheritance, as in many of the "economic" or performance attributes, and their expression is often affected by environmental factors; the best proof of the genetic make-up of an animal lies in determining what he or she has passed on to its offspring. With special regard to performance characters, major difficulties arise in defining, first, the particular kind or level of performance required, and, secondly, the extent of the environmental influences—how much of the particular performance is attributable to feeding, health, status, housing conditions, management, etc., and how much to inheritance? In other words, what proportion of the variation in performance is due to genetic effects as compared with that due to the interaction of genotype and environment? Various methods of estimating the "heritability" of characters have been developed and applied to a wide range of characters under a great variety of conditions.

The estimates of heritability have shown great variability in themselves: a series of reviews recently published in the *Journal of Animal Science* (1958) by Warwick (beef cattle), Terrill (sheep) and Craft (pigs) indicate the ranges of estimates found in the different species and types of stock. In spite, however, of the ranges in values, the various characters can be broadly considered as to whether their heritabilities fall into low, medium, or high groups, and thus some idea obtained as to the relative importance to be placed on them in selection, and to the selection methods needed to lead to improved performance in the offspring. (It must be emphasised, however, that much more information is needed on the degrees of genetic correlation between performance characters themselves so that there may be greater confidence in knowing that selection for one may give either positive or negative response in another). For example: in dairy cattle, milk yield is of low heritability compared with fat percentage; in beef cattle, birth weight, rate and efficiency of gain after weaning are more strongly inherited than conformation grade at weaning, and while dressing-out percentage may be highly inherited, carcass grade is not; in pigs, lengths of body and of legs are high, conformation score low, numbers of pigs farrowed and weaned and weight of litter at weaning generally low, weight at 5–6 months and economy of gain relatively high, and, compared to all these, carcass measurements such as length, eye muscle area, thickness of back fat and percentages of cuts (based on carcass weight) relatively very high; in sheep, among the characteristics in the high group are face covering, staple length and fibre diameter, in the moderate group, weights at birth, weaning, and 1 year old, fleece weight and wool yield, and milk production, while among those relatively low are type of birth (twins or multiples), type or body conformation and condition.

The basic conditions for any method of selection are that either the character should be discrete and clearly recognisable, as in the cases of simple qualitative characters such as coat colour, etc., and of many recessive defects; or that it should be measurable in reasonably accurate terms, as with "economic" performance traits. More studies are needed on appropriate methods of assessment and stricter definition of the precise attributes which make up economic performance worth. For example, while milk yield and fat percentage are fairly straightforward, solids-not-fat is not; while the desirable characteristics of a bacon pig may be reasonably definable, those of a heavy pig are not, as yet; in the evaluation of beef carcasses, objective assessments are obviously needed, but a recent study (Bodwell *et al.*, 1959) shows marked differences between observers and between sides of the carcass, and that some measurements, such as those on the tibia, are practically useless.

In so far as progeny tests are concerned, the general conditions under which they can be helpful and the scale necessary to give reasonably significant results have become fairly well recognised. In the simpler cases of recessive defects likely to increase in fre-

quency where particular sires or families are favoured in a breed, the three main forms of test for possible carrier sires are: mating to recessive females, to known female carriers, and to their own daughters. Johansson and Robertson (1952) showed that for reasonable accuracy (one chance in twenty that the heterozygous sire should not be detected) at least 5, 11, and 23 offspring are needed respectively in the three kinds of test; the last has the additional value that it brings in all possible recessive defects. For pigs, King (1955) concluded that a boar could be effectively tested for recessives by mating him to five of his own daughters, needing only one test litter from each. He also critically examined the circumstances under which progeny tests can be most valuable, as stated by Dickerson and Hazel (1944), i.e., when (1) the test would involve only a small increase in generation interval; (2) the females have a low reproductive rate; (3) the character concerned is of low heritability or is expressed only in one sex; or can only be assessed on slaughter; and (4) the breeding unit is numerically large. These conditions impose severe practical limitations: (1) implies a rapid turnover of breeding stock and early testing; (2) a high replacement rate in the breeding population; (3) difficulties in eliminating or discounting environmental effects; and (4) forming larger individual units, by co-operative breeding programmes, or by spreading artificial insemination projects.

The idea that progeny testing is essential in respect of characters of low heritability has become popular: to see it in perspective it is necessary to examine the methods used, their limitations of application and interpretation, and its relative merits compared to other tests that might be available. Difficulties of character appraisal arising from environmental effects have given rise to two main forms of testing procedure. In the first, samples of the progeny of different sires are assembled at testing stations under "standard" conditions of management, feeding, housing, etc., assumed to be highly repeatable from year to year and from one station to another, and to give conditions appropriate to reasonable performance. In the second, larger groups of offspring are recorded under farm conditions and compared with other samples of the whole population, it being assumed that management and environmental effects are thereby randomised and can be neglected, especially if the groups are compared with their contemporaries. There appears little evidence as yet as to how far the foregoing assumptions are valid. Differences between pig testing stations have been revealed, as well as seasonal differences; and work such as that of Burt (1955) and Wiener (1959) on differences in cattle herd environments strongly suggest the need for further studies before it can be considered that such influences can be randomised, at least under British conditions, and that "contemporaries" dispersed over wide ranges of environments are of equal or similar physiological status. Moreover, the effects and distribution of intercurrent disease clearly need closer examination.

Testing stations are most widely used for pigs and dairy cattle. In Great Britain, the National Pig Progeny Testing Board has now taken responsibility for continuing and extending the tests formerly carried out by commercial interests and the N.P.B.A. and operates five stations; for dairy cattle, the B.O.C.M. organisation started bull progeny testing in 1953, in co-operation with breed societies, and the M.M.B. have recently had progeny of beef bulls mated by A.I. to dairy cows tested through the co-operation of the N.A.A.S. Experimental Husbandry Farms.

The testing station is expensive to run, especially since it can test only a small sample of the sires being, or likely to be, used. But this disadvantage must be viewed in the light of the use made of the results; for example, it is reduced if the tested sires, and sons of the best of them, can be exploited either through breeding centres or groups of herds, or through A.I. In Denmark, concentration on the descendants of tested boars has certainly contributed to the improvement of the Danish bacon pig, and the very great majority of bulls now in use in A.I. are sons of sires tested in the Danish stations. On the other hand, testing station results appear to have made little impact on the Canadian pig breeding industry. Also, inherent difficulties occur in securing the progeny sample for test: four piglings per litter may not be free from bias in selection by the breeder, groups of 15 or more heifers to be of the same age at calving and calving close together further restrict the choice of sires to be tested. Moreover, it is not yet known how far pre-test rearing conditions affect test performance, nor whether the test environment may give rise to genotype-environmental interactions different from those occurring where the other offspring of the tested sires are kept. With regard to pig testing stations, King (1955) considers that differences due to pen position, between stations, and between seasons of the year may best be overcome by statistical methods of control and comparing group results with their contemporaries at each station rather than by attempting to get more complete control of the environment. Similarly, attempts to standardise rearing conditions for calf progeny groups would increase the expense and decrease the throughput of the stations and still not overcome the same objection. Yet data from testing stations add materially to the information available on the development of the characters themselves and so can be of value in other forms of performance testing, such as sib-testing, particularly for characters which can be assessed in the live animal, without slaughtering. In any event, progeny testing station results can provide not an absolute evaluation of the sire's worth, but a basis for comparison between sires.

The second method, of contemporary comparisons, depends, obviously, on there being adequate field records available from widespread performance recording schemes, as in dairy cattle. When more farm recording schemes are further developed, as for pigs, and possibly also for sheep and beef cattle, modified forms of this test may become practical possibilities. This method, especially in Great Britain and New Zealand, has replaced the older ones of daughter-dam comparisons, as still used in the United States for example. The background to the New Zealand "sire survey" lies in the relatively simple, seasonal, dairy farming system based preponderantly on pasture, with calvings mainly in early spring. The results are in terms of butter fat yield and the sires' evaluations

are based on an "expected daughter average". The actual yields are adjusted by age corrections to mature equivalents and then related to herd, contemporary daughters of other bulls, and district averages. In effect, a bull's survey results show whether, and by how much, his daughters may differ from those of the average bull. The "preparatory survey" is based on at least six but less than ten daughters, the "official survey" on at least ten daughters. In the final rating of sires, adjustments are also made for the numbers of daughters included in the surveys of each bull, and the results are published in Sire Survey Registers; the fifteenth edition (1959) gives results for over 3,000 bulls. It is stressed that these surveys are only a guide to breeding worth and must be considered in relation to individual farm conditions (New Zealand Dairy Board, 1959); their use is mainly by breeders as an aid to selection of sires.

The British system was developed primarily to assess the breeding qualities of bulls used at artificial insemination centres. The derivation of the Relative Breeding (or Genetic) Value (Milk Marketing Board, 1953; McArthur, 1954) is based on the assumption that the bulls have been mated at random in the herds where used and that the daughters' contemporaries are of average genotype. First lactation yields are used, without correction, the essential data used being daughters' and contemporaries' averages, the herd average and the breed average. Allowance is made for regression of future daughters on present test daughters and for the heritability of herd average yields (at 20 per cent.). Here the emphasis is on "*Relative* Value". The clear distinction is between the high and the low value bulls; discrimination between bulls around the 100 value cannot be expected.

Comparison between progeny test station and field results has now been possible for 16 Friesian bulls tested in four groups of four bulls in four years (M.M.B., 1957/8); the progeny test station results could discriminate between the extremes of good and bad sires as judged in the field, but not between those which fell in the intermediate category, and thus cannot be closely relied on in forecasting sire performance in practice. This conclusion generally supports that drawn by Robertson and Mason (1956) from results in Danish testing stations.

This discussion suggests that where performance is measurable, systematic progeny testing can provide guides to more accurate selection, as well as data on the performance characters themselves, on techniques of assessment and the heritabilities of the characters concerned. The same data can serve to establish more clearly the circumstances under which progeny testing is helpful and justified, or where it can be replaced by other tests. For example, many economic performance traits in pigs, such as rate of gain and back fat thickness, can be assessed in the live pig; where such traits are highly heritable, the individual performance test can be more rapid, cheaper and as accurate as the progeny test. Hence, boar performance testing is spreading as a selection aid. Similar situations may be envisaged with regard to other forms of production, e.g., beef, mutton or lamb, and wool, and where the production of economic performance is based on crossbreeding systems. This prospect is enlarged when it is recognised that in any interpretation or application of any test results the breeder is confronted with other considerations. From his point of view, the fertility, viability, longevity, hardiness, etc., and "type" of his stock are important; compromises between high performance in some traits and low in other physiological or anatomical characters have to be made to arrive at some form of overall worth index, or total score, selection for which will slow down progress in any one direction. These other characteristics may even be incompatible with "economic" performance, especially in the female stock with which the breeder is most concerned; they may affect the survival or functional size of his breeding unit, so cannot always be neglected in favour of sale products, even though they have as yet to be assessed subjectively.

This last comment raises some important issues. The search for precision has led, in some quarters, to the complete neglect of any procedures involving crude or subjective tests, although in practice, and over long periods, improved production has been achieved by their use; as a result, it has been overlooked that the less precise assessments, for example, of fleece characters in the great wool producing countries as in Australia, New Zealand and South Africa, have in fact given tests of breeding policies which breeders have used to attain more efficient production, and that carcass gradings of fat lambs have enabled breeders to appraise their breeding and husbandry systems and achieve high performance standards. Certainly, the development of more objective and critical assessments, now actively progressing, will give more accurate selection, and replace the older methods, as, for example, is happening with fleece measurement as opposed to visual and tactile appraisal in sheep and wool classing, yet it remains to be seen how far they can speed up overall practical improvement. Again, while it may theoretically be advisable to reconstitute the structure of a livestock industry so that specialised breeding units or groups contribute stock to large "commercial production" units, as in specialised crop or poultry production, it must be remembered that the size, scope and continuity of the herds and flocks of the larger farm animals are determined by geographical, edaphic and sociological considerations as well as by economic conditions. This being so, it may be argued that there is as much or more need to seek effective selection methods which can be used in the existing circumstances as to aim to reorganise and rationalise the structure of the breeding industry.

References

BODWELL, C. E., EVERITT, G. C., HARRINGTON, G., and POMEROY, R. W. (1959). *Animal Production*, **1**, 51.

BURT, A. W. A. (1955). *J. Agric. Science*, **45**, 389.

CRAFT, W. A. (1958). *J. Animal Science*, **17**, 960.

DICKERSON, G. E., and HAZEL, L. N. (1944). *J. Agric. Research*, **69**, 459.

JOHANSSON, I., and ROBERTSON, A. (1952). *Proc. Brit. Soc. Animal Prod.*, 79.

KING, J. W. B. (1955). *An. Breed. Abst.*, **23**, 347.

MCARTHUR, A. T. G. (1954). *Proc. Brit. Soc. Animal Prod.*, 75.

MILK MARKETING BOARD (1953). Report Production Division No. 4.

——. (1957/8). Report Production Division No. 8.

NEW ZEALAND DAIRY BOARD (1959). *Sire Survey and Merit Stud Register*, fifteenth edition.
ROBERTSON, A., and MASON, I. L. (1956). *J. Agric. Sci.*, **47**, 376.
TERRILL, C. E. (1958). *J. Animal Sci.*, **17**, 944.
WARWICK, E. J. (1958). *J. Animal Sci.*, **17**, 922.
WIENER, G. (1959). *Animal Production*, **1**, 61.

THE SPEAKER'S INTRODUCTION

The Chairman, Professor J. A. Laing, introducing the author, said that this was a subject which had been almost as neglected by the profession as their last topic, that of blood grouping in domestic animals. The author had made a special study of genetics in domestic animals. He was an international authority on the subject and they should be very grateful that he had come so far to present his paper.

Professor J. E. Nichols, presenting his paper, said that he proposed to take the very simple and straightforward aspects of progeny testing and try to place the subject in some perspective in relation to the livestock population of the country, which was what they were interested in. They all knew that the proof of the pudding was in the eating. To change that old saying, he would say that the proof of the breeding was in what was begotten. That quite clearly summarised the position with regard to progeny testing.

There was nothing new about progeny testing. For a long time it had been a recognised method by leading breeders of livestock, but in recent years closer study had been made of the methods and objectives. The sum total of the tremendous amount of work on testing and tests which had gone on in every stockbreeding country in the world was to try to make the methods of testing more precise and accurate and to establish the conditions under which progeny testing could and should be used.

He emphasised that progeny testing, with all due respect to the pundits, was becoming a bit too popular. It was merely one of the methods available to breeders to enable them to select the animals, mainly the sires, to be the parents of the next generation. It was so popular that many of the other possible methods of selection were being rather pushed into the background.

There was increasing evidence that, for many practical purposes, progeny testing may be too slow, cumbersome and expensive and that it might be done under conditions which were in no way simulated in practice. Because of that, breeders might be putting themselves into a somewhat misleading position.

The simple matter was that characters of stock fell into two broad groups. There were the simple, straightforward ones, and in the other category were the more complex economic characters—the performance characters. Some of these, such as milk yield, could easily be measured, and some such as hardiness could not. It might be said that longevity could be measured in terms of the years that an animal lived, but if the animal was a female what was of importance was not the length of life but the amount of milk she gave and the number of offspring she had. There were considerations of that sort which remained to be assessed.

The significant point was that these economic characters were those which were affected by the environment, in the broadest sense of that word—the way in which the animal had been raised and nurtured; the whole problem of disease and everything else. Because so many of these economic characters were so grossly affected by environment, they had this problem of how to dissociate the environmental effects from the inborn inherited effects.

Recent studies had been made of these complex economic characters to determine what was known as "heritability". This was not an easy concept to put into a few words. In effect, it was that the heritability of an economic character was the measure of the amount of the variation in that character which was attributable to genetic effects. There was increasingly clear evidence that a lot of the economic characters, especially those of conformation, of growth rate, of meat and of the fleece, were fairly highly inherited, whereas milk yield and other characteristics of that sort were rather lower in heritability.

There was plenty of evidence that in the case of characters of low heritability—he took milk yield as one—some form of progeny testing was necessary to evaluate the worth of a sire; but with regard to many of the other characters—meat, growth rate, and so on—where heritability was relatively high, there was strong evidence that, from the breeder's point of view, progeny testing could be relatively a waste of time. That was why there had been a swing over in many parts of the world, especially in America, and it was happening now in this country, to consider that for things like pigs, the performance test of the boar was, for practical purposes, a more direct test, and possibly just as accurate, as a progeny test applied to his offspring. It was his conviction that at best progeny tests, in any controlled sense, could only be applied to a very small fraction of the sires which were likely to be at work in any livestock population.

Performance testing for boars, and even for beef bulls, was just as direct and it was quicker and just as useful as an indication of the prospective merit of the sires.

Because of these environmental considerations for many of the economic characters, two methods had been developed for carrying out testing. The first method was that commonly practised in many parts of the world. It involved collecting at some central station or stations the offspring of particular sires, in groups of 10 to 15 for heifers, and exposing them to strictly controlled conditions of housing, feeding and everything else. These testing stations had contributed very useful results, but at best they did not, and could not as he saw it, provide any absolute evaluation of the breeding worth of the sire from sample groups collected at central stations.

First, one could not really control environment in such an absolute sense for the major kinds of livestock. On the other hand, much depended on the use made of the sires by the breeders. In that sense, it was probable that the Danes had led the world, in that they had geared into their progeny testing station activities the use of approved herds in which the progeny tested sires or their sons were used. But that was, as it were, imposing by co-operation a certain form of control or restriction upon the whole breeding industry. That had worked in Denmark. Whether or not it would work in other countries was doubtful. It had certainly not worked with pigs in Canada. Progeny test results had made no impact whatever on the Canadian pig breeding industry.

The other method was to carry out field surveys, such as they had in their well-known dairy bull progeny testing as carried out by the Milk Marketing Board. The records of the animals were taken under ordinary practical field conditions. It was assumed, probably quite rightly for statistical purposes, that that randomised the environmental effects and, because of that, it was claimed that if heifers by a particular bull were compared with their contemporaries in the same herds, then a fair, relative evaluation of the sires could be made. However, the difficulty was that the breeder who was going to use a progeny tested sire, or a son of that sire, was still left with the problem of how far his own environment as at all average or normal, or how it compared with the environments of his neighbours in the same county.

He wanted to leave them with the thought that it was in connection with the study of the environment within a particular herd, a particular flock on a particular farm, that much more work was needed. As he saw it, in Great Britain they were committed to an agricultural system in which, for the most part, herds of cattle were small and even flocks of sheep were fairly small. The problem for the individual breeder was that he had to consider his own breeding unit and his own farm environment as the first consideration before he started to have any definite breeding policy at all.

One would like much more information about that so that individual breeders would be able to make compromises, to measure and assess their own requirements in their own herds and flocks far more accurately than they could at present.

Progeny testing was all right in certain circumstances. It was all right as a general method to be done on a large scale, but ultimately what mattered was the use made of it which had to remain the responsibility of the individual breeder, who for the most part had only a small breeding unit in which the female characteristics of his stock were of first importance. One was often tempted to wonder whether or not, in the progeny testing schemes which were being so widely applied, that point was being overlooked and the buck being passed to the breeder himself. Singularly little was being done to help him to evaluate his own herd or flock environmental problems.

The Opener

Dr. J. O. L. King, who opened the discussion, said he supposed that most of them had spent a pleasant summer evening leaning on a gate beside a farmer client looking at a bunch of heifers. They had listened to the singing of the birds, they had breathed the scented air of the countryside, and they had delighted in the peace of their surroundings. They had admired some of the heifers and criticised others, and they had honestly felt that they had been helping their clients in the selection of stock by appearance.

It now appeared that, however much they had enjoyed themselves, they had been wasting their time and the time of their clients. Now, if their advice was sought on such a matter, they met the farmer in his office, with the ringing of the telephone as a substitute for the singing of the birds. They had to look at milk records and other documents propped up on the unopened accounts and seed catalogues usually to be found in such a room. The only possible advantage to them as advisers was that the supply of liquid refreshment was handier.

Success in breeding high quality individuals was relatively common. Success in breeding high quality flocks and herds consisting only of superior individuals was most uncommon. All breeders had as their aim the production of a superior strain. It seemed at the moment that the shortest line between the average herd and superior herd was along the line of progeny testing. That was common sense; but, as common sense was not quite as common as it might be, they were fortunate to have present a man of the eminence of Professor Nichols to address them on the subject of progeny testing. They must all be grateful to him for preparing such a concise and clear paper covering as it did work carried out in many countries, and for sparing the time to join them at this Congress.

As Professor Nichols had said, progeny testing was a method of breeding that was very much in the mind of the farming community. It marked a real advance in animal husbandry. Because of its importance, it was essential that progeny tests should be carried out properly, so that only true evidence was given. In the early days, when progressive farmers were progeny testing their own bulls by the daughter-dam comparison method, the fact that the daughters were being more highly fed than the dams had been, was in some cases overlooked, and certain sires were credited with increases in production which were entirely due to improved standards of management. Such mistakes were not likely to be made now, but he emphasised the point made by Professor Nichols that a representative group of progeny must be tested.

Professor Nichols had referred to the selection of the 4 pigs from a litter to be sent to the progeny testing station by a breeder, and at some centres the heifers to be tested were being selected on type before they were sent to the centre. Any such selection greatly reduced the value of the test as although, as he had indicated in his opening remarks, it was difficult to select by inspection the best among a group of good animals, it was possible to pick out those of poor conformation which would never become good producers. If an animal sired even a small number of inferior animals, that fact should be recorded and not concealed.

Another point of importance if progeny test results were to be widely used was that as many economic factors as possible should be assessed. In dairy cows, concern was being expressed at the low percentage of solids-not-fat contained in the milk of certain herds. Although feeding was of importance in maintaining a high level, breeding must be the long-term method of improvement. Bulls that were being progeny tested by the relative breeding value method outlined by the author, were being tested for yield and fat only. Although a good fat yield was generally an indication that the solids-not-fat content was also reasonable, this did not always follow; and, if widely used, a bull producing daughters yielding milk low in solids-not-fat might do a great deal of harm before the damage that was being done was realised.

He also wished to mention the possible value of the progeny-tested female. Breeders of Thoroughbred horses had frequently observed that certain mares produced high-quality racehorses irrespective of the sires to whom they were mated. Cattle breeders had made similar observations, that some cows would produce a succession of high-yielding daughters by different bulls. He wished to ask Professor Nichols whether he felt that more consideration might be given to the selection and use of progeny-tested females. The practical difficulties involved in testing females were well known, but might not breeders attain a higher standard if they mated progeny-tested males with progeny-tested females wherever possible?

They, as veterinary surgeons, while interestsed in progeny testing in all its aspects, were chiefly concerned with any effect which its widespread use might have on the health of the animal population of this country. In the case of cattle, a suggestion had been made that 200 bulls might be sufficient for the insemination of 2 million cows. Would the intensification of certain blood lines which this might cause in 25, 50 or 100 years' time be likely to have any deleterious effect?

Many inherited lethal and semi-lethal characters had been reported. Various writers had compiled lists of them. They would constitute no special problem if there were some simple method of detecting their presence in the carriers, but in the vast majority of cases in farm animals there was as yet no method of recognising the presence of these genes in the carriers. In some cases they could be discovered by a careful examination of an animal's pedigree, but that method was not conclusive and it might well lead to the exclusion of a potentially valuable animal.

The only reliable way of detecting such carriers was by progeny testing and, as Professor Nichols had pointed out, the only form of progeny testing likely to pick out all possible recessive defects was the mating of a sire to his daughters, in which case at least 23 offspring would be needed. Did Professor Nichols consider that such a test was necessary before a sire was widely used, or did he consider that the risk of spreading undesirable genes was not sufficiently great to warrant its use?

He also wished to mention the possibility that progeny testing might be extended to selection for disease resistance. Plant breeders had evolved disease-resistant strains of plants. Poultry breeders had shown that it was possible to breed strains with a marked resistance to leucosis. In theory such a process was possible with cattle. For example, it might be possible to produce strains that were resistant to mastitis. Such resistance would help to control the condition, for even if the resistance was not absolute, it seemed probable that the genetically resistant animals might provide a better medium for the successful treatment of the condition by antibiotics. Such a plan would probably break down in practice because few breeders would be in a position to provide the necessary exposure to the disease needed to test the resistance of the strain but, as they were thinking of future developments in progeny testing, he would like to hear what Professor Nichols had to say on the subject.

The General Discussion

Mr. G. F. Smith (M.M.B., Wadhurst): "You catch me somewhat unaware, Mr. Chairman, when you ask me to contribute on the subject of progeny testing; however, let me first congratulate Professor Nichols and Dr. King on their remarks, and then perhaps I may clarify a point raised by Dr. King. The Board are at this very moment testing for s.n.f. the daughters of many A.I. sires and this particular factor is exercising in no small measure the minds of those responsible for this work.

"The Board's progeny testing programme is based on a sound system of recordkeeping and semen utilization in the belief that by putting a good number of young bulls in at the bottom, a few will reach the top of the ladder; in fact, based on present experience, perhaps 1 in every 4.

"If we are to accept the fact that progeny testing is fundamental to livestock improvement, this procedure must, in fact, continue, and ultimately it may well be possible to provide an A.I. service based on the proven sire, and the young sire who will carry out a limited number of services and then be rested. At the present time this ideal situation is becoming quite a difficult thing to achieve, especially in breeds comprising small cattle populations. During the remarks of the foregoing speakers, doubt has been shed on the desire to reduce bull strengths which, in fact, means increasing the services by each good sire; fear has been expressed that undesirable recessives may well be introduced and spread over large sections of the cattle population. It has been advised that sire-daughter matings should be carried

out in all cases. Already we have carried out a pilot experiment in the case of 25 young sires, and although we did not reach a maximum number of some 21 matings in all cases, we did not find that the inbreeding was more valuable than the reporting scheme already in use among our field staff.

"Finally, through you, Sir, I would like to ask Professor Nichols if he could outline any plan which might be adopted by small herd owners to progeny-test a bull adequately."

Mr. F. A. Crowhurst (Maidstone): "Does Professor Nichols think that in the study of progeny testing in farm livestock sufficient attention has been paid to an experiment in progeny testing which has been going on for 200 years, i.e. the Thoroughbred breeding industry in this country?"

Mr. J. Moss (Bromsgrove): "I think we must all agree that progeny testing in some form is necessary to measure small differences in the heritability of desirable characters. It will also seem obvious that for the major characteristics such as milk and butterfat production, and the more obvious points of conformation, the Relative Breeding Value Method is more reliable than the Danish Method or the test in a private herd. But we should recognise that the other, less definable characters, which make livestock breeding and animal husbandry a way of life more attractive than commerce will always be a saleable commodity.

"It is the use we make of progeny testing that is important. The aim should be to test for the right characters and then to make the most prudent use of the sires so selected. I am worried by the ultimate effect of mass breeding. The 200 bull to 2,000 cows status has been mentioned. I know that the Milk Marketing Board has recognised the possible dangers of this state of affairs being reached and that they have posed the question of its effect on the future to leading geneticists and others throughout the world. This is a grand opportunity to hear first hand Professor Nichols' views on this problem. Are the steps taken to spot lethal recessives and abnormals enough to safeguard us for the time when blood lines have been so reduced that some of the less easily recognised weakening factors may become more important? It is a problem that we should face up to squarely now.

"The bird populations and those of other species which have been raised from one pair, and the regeneration of almost extinct species has been used as proof that inbreeding need not be harmful. But it must be remembered that here natural selection and survival of the fittest has also been operating. We must be careful that we do not select a strain of cattle that requires a highly specialised environment to exist."

Dr. W. R. Wooldridge (London): "We have heard a great deal about what we might call the positive or cheerful side of breeding but little on the black side.

"Whilst it is obvious that one should, in order to understand more fully the many factors involved in heredity, carry out experiments in breeding from those animals carrying good qualities, nevertheless it seems to me that we are neglecting the equally important side of the subject involved in breeding from those carrying bad factors. It is the normal practice amongst breeders to cull at an early age those which are not considered to have the necessary qualities for improvement of the breed. These culls are usually slaughtered or at least prevented from breeding. Surely it is important to breed from these culls to see what other factors are thrown up as a result?

"May I ask Professor Nichols whether work of this character is in fact being carried out and how it can be developed?"

The Reply

Professor J. E. Nichols, in reply, said that attention had been given to the testing of racehorses but, speaking quite personally, he thought it was an extremely awkward industry to look into from this point of view. It was doubtful whether in the Thoroughbred racehorse breeding industry any sire was sufficiently randomised in his matings to enable one to have any clear indication of his real worth. It could be argued by the Thoroughbred breeders that the test of the racecourse was adequate, but how many of the offspring of a particular sire, who may be very restricted in his use, were subject to really critical and comparative racing tests? He thought there were very few indeed.

He commented on the scale of the testing schemes of the Milk Marketing Board and said that the time might come when a similar impact might be made on the pig population but, as far as beef cattle and sheep were concerned, they were still rather a long way from that. He emphasised a point that had been made indirectly by Mr. Smith. The information available to the Milk Marketing Board through its recording scheme was probably the most valuable source of data available to the Board.

As to the possibility of the small breeder carrying out a progeny test of a bull, the answer was that it was almost nil in the ordinary herd of the ordinary size; but, to revert to a point made by Dr. King, he thought that the small breeder could be encouraged to play a more important role than he had done in the past by aiding in the search for the superior female. In the smaller herds they could look for the reservoir, as it were, of superior cows from which the future superior bulls could be bred.

The question of the progeny-tested female was very difficult. It had always been a matter of considerable interest to him that in the Australian merino stud flocks, which were among the biggest breeding enterprises in the world, there was great stress on the proven female lines. Most of their leading rams were from particular ewes or female lines. There was a possibility of greater emphasis being placed on the female side.

Another point of interest was that, even in a herd of dairy cows, very frequently the breeder would say, quite honestly, that he owed the success of his herd to the fact thet he used bull A and followed him by bull B. If the position was examined closely, one found that that was just nonsense. What had happened was that the first offspring of one of his foundation heifers were two daughters. They were kept in the herd because the farmer wanted replacement heifers. The first two offspring of those two daughters were also daughters, so he had seven females all related to one foundation cow. The influence of the bull was negligible. That kind of circumstance was very often responsible for the structure of a herd. On going through the breeding structure of many different herds, he had been astonished to find how often that was the case.

Professor Nichols agreed generally with the point made by Mr. Smith that, according to available evidence as far as it could be determined, no marked difficulty would seem likely to arise from the restriction of the number of effective sires. On the other hand, he confessed that he had a great deal of sympathy with Mr. Moss's point of view. He felt that one of the big dangers in all livestock breeding policies in Great Britain was that of making each breed more uniform and losing a lot of the genetic variability which was in the breed. That was heresy if they liked, but it was certainly his feeling!

He could not see any terrific advantage in having a large number of animals of one particular breed, all to one pattern, so that the breed as a whole was too uniform. They might be getting to that stage with some of the numerically smaller breeds. Studies which had been made confirmed that in some of the numerically small breeds—take the Aberdeen Angus as an example—there was always a lot of out-crossing of strains; there was a sort of reservoir of genetic variability held as such in the breed. That was an advantage in any breed. One would not like to see that variability completely lost by a restriction of blood lines within a particular breed. That was something which the authorities, such as the Milk Marketing Board and the non-Board centres, should bear very closely in mind.

Mention had already been made of the Thoroughbred racehorse. As far as he knew, there was a lot of out-crossing of strains going on in Thoroughbreds. That maintained within the breed a certain amount of flexibility.

Mr. Moss had spoken about planning for 100 years ahead. Was that worth while? They in their profession, and even he in his, were always confronted with one grave difficulty. The recognised breeder, the fellow at the top of the tree, wanted things to stand still. He had a vested interest in that more than in where the breed was likely to go. On statistical grounds, the general view was reasonable that in numerically large breeds there really was not much point in using a large number of sires. When they got to the stage of 200 bulls for 2,000 cows, he would like to know what those 200 bulls were to be. If they were talking in terms of dairy cattle, were they going to be Friesians? Were 150 of them to be Friesians? He did not propose to go into any breed controversy over that.

Mr. Moss had made another point which was absolutely right. The small breeder was concerned, and rightly so, with a lot of other characteristics. He did not restrict his interest only to those which were measurable. He had to have room for manœuvre with regard to those other characteristics as well.

The author fully agreed that a lot more attention should be given to the question of disease resistance. Dr. King had mentioned mastitis in dairy cattle. The Milk Marketing Board, through its field officers, was in a position to get a lot of data, and so were veterinary practitioners. He looked forward to the time when there would be a whole mass of information about disease resistance. This could be codified and used in the selection of disease-resistant strains.

In reply to Dr. Wooldridge, the author pointed out that many of the husbandry, feeding and disease control methods that were applied were empirical. Often they did not know much about what on earth they were controlling or why.

He agreed with Mr. Wright that the time had come when they wanted to know a great deal more about broad environmental effects. It was only with pigs and poultry that any farmer could exert a rigid control of the environment of the stock during the whole of their life. The rest were exposed to other environmental conditions which altered between one part of the country and another. It was high time that more information was collected on environmental effects, especially, on the ruminant group. Changes occurred as between Scotland and Cheshire and as between Cheshire and Essex and in different parts of Wales. These changes were real but they were not easily measurable. He thought that attempts should be made to measure them so that they could be assessed. One difference between the relative breeding value method in this country and the survey testing schemes in New Zealand was that in the latter place greater attention was paid to the normal environmental circumstances of the cattle. They had to make those adjustments.

Someone had mentioned doing a lot of work in the office of the dairy farm. There was one respect in which the practitioner and the farmer could go a long way in assessing the farm environment of the dairy cows. There was not a great deal of work involved. They should pay close attention to the lactation curves of the individual cows, or even the herd as a whole; close study of them could pin point all sorts of environmental effects. They were highly important to the individual farmer.

He was very happy that the points raised during the discussion had underlined some of the difficulties of interpretation and use of this one particular method of selection. Some of the comments made by Dr. King had already been touched upon. He would now deal with one or two of the other remarks which Dr. King had been good enough to make in opening the discussion. Dr. King had made the point, which was emphasised by Mr. Smith, that the question of inferior progeny, when they occurred, should be recognised. They all knew how often inferior progeny were concealed. That attitude of mind had to be cured. That was where the practitioner could be of enormous help in the whole scheme of things.

There was one famous breed of dairy cattle which had spread enormously but had recently undergone some recession. Ten years ago everybody who had these cattle knew that certain deformed calves were turned out, but it was absolutely forbidden to mention them. Now, within a space of 10 years, that breed was quite open about it, and breeders did not conceal as often as they used to the birth of deformed calves. The result was that most breeders knew the sort of blood lines in which it was likely that the trouble would arise. They were reducing the frequency of that deformity simply by not using carrier stock. There was still plenty of it in the breed, but now that they had got over the attitude of secrecy a general improvement was being brought about.

Dr. King had also referred to the possibility of the detection of carriers. That was a highly important aspect, and a lot of work was being done on it. Dr. Sinclair had referred to blood studies. He was sure that in time they would aid in the detection of carrier animals or some of the disadvantageous characters.

He concluded by thanking Dr. King for the way in which he had opened the discussion.

Blood Grouping in Domestic Animals

BY
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Introduction

IN the nineteenth century it was known that animals which had been infected by certain bacteria acquired the ability to resist further infection of the same kind. When a suspension of these bacteria was mixed with serum from recovered animals the bacteria were sometimes seen to clump together or agglutinate. This depended on the combination of antigens of the bacterial cell with antibodies in the serum and was a technique already in use to study problems of this nature. In 1900 antigens which were found on the red cells of human beings and of goats discriminated between individual members of each species. In 1910, Todd and White noticed that the serum of cattle which had received injections of bovine blood would react with, and dissolve, the red cells of all other cattle which were tested. Furthermore they mixed a sample of this serum with the red blood cells from one cow and then removed them. As a result the serum lost its power to react with a second blood sample from this animal yet it could still react with blood from other cattle. This serum or reagent would then discriminate between this one animal and all others.

At this time biologists interested in heredity were on the look out in all species for clear-cut differences which might be under genetic control. The human blood groups were soon found to be models of this kind. Fisher in Britain and Irwin in the United States saw that blood grouping need no longer be confined to the field of therapeutic blood transfusion. They had the foresight to see that the red blood cell probably possessed a large number of easily recognised characters under strict genetic control and that these would provide a rich source of material for the study of genetics in man and animals.

In animals, blood grouping techniques do not yet offer a rational basis for blood transfusion therapy as in man. They would, however, help to avoid some of the dangers of blood transfusion, particularly in the dog. Haemolytic disease of the new-born foal and piglet can be better understood and avoided by a knowledge of the consequences of blood group incompatibility. The cattle breeder can use the strict laws of blood group genetics to clarify questions of pedigree, the diagnosis of twins and the detection of the freemartin. The number of blood factors we shall consider is becoming so great that their hereditary nature and variety are attracting the attention of the genetics. To these investigators blood grouping is no more than a technique to identify the presence of genes as indicators of genetic constitution.

The characters with which these methods are concerned are not graded in their expression like the weight of an animal. They are qualitative characters which are clearly present or absent, or in an otherwise distinguishable form. This property permits definite statements to be made about the genetics of individual animals. Blood group characters are not visible like horns and polled, and they cannot be consciously chosen or rejected for his stock by the animal breeder. In the living animal they cannot be altered or removed or acquired in any way. At present the greatest variation in the blood of all species of domestic animals is to be found in the antigenic properties of the red blood cells. These can only be detected by the effect of their combination with the specific antibody with which each corresponds.

Apart from the antigens, discrete qualitative variants of other blood constituents can be found. The haemoglobin in adult horses, cattle, sheep and goats exists in at least two molecular forms, one of which carries a higher surface electric charge. Under the influence of an electric current these two forms migrate at different rates and can thus be identified. A different technique of this method has also disclosed individual gene-controlled differences in the serum beta globulins of cattle and sheep. Also in sheep, remarkable clear-cut differences have been found between those with a high and those with a low concentration of red cell potassium. These recent discoveries encourage the view that the extension of biophysical and biochemical methods will disclose the existence of gene-controlled physiological variants of many body constituents. There is no doubt that they will be useful instruments in the genetical investigations of domestic animals.

Methods

A blood antigen can be detected only as a result of its combination with specific antibody. These antibodies like those against micro-organisms, occur in the serum of animals. Naturally occurring antibodies are those found in animals which have never been injected with the corresponding antigen. They occur in all species of domestic animals, are usually weak and in each species no more than one or two are of frequent occurrence. Those antibodies which most frequently occur in people and animals lacking the antigen are the human, A B H and P; the cattle J; sheep R and r; pig A and O, and horse A and O. With the human Le^a these antibodies and their antigens have complex serological relationships and other similarities which set them apart from the others, and comparative studies are beginning to elucidate the nature of these relationships. The immune antibodies are those which appear in response to the stimulus of foreign antigen. Nearly all the blood group antibodies have this origin, usually the donor and recipient are of the same species. Antibodies vary greatly in the regularity with which they can be induced. In all species studied some antibodies can

regularly be stimulated to high titre. No significant influence on antibody formation has been found for breed or season, and in general the production of blood group antibodies is a matter of trial and error (Braend, 1959).

The Serological Basis

The red cell antigens can only be detected after they combine with the antibody to which they correspond. The usual analogy offered is that of a lock which can be opened only by the key to which it corresponds uniquely. The analogy is closer to truth than this for many locks will be opened by keys which were not intended for them but which have an appropriate conformation. In some antigen-antibody reactions the same lack of exact specificity occurs so that antibody preparations will react with antigens which have a slightly different genetic control. Cross reactions of this nature do not seem to occur between antigens which are controlled by widely differing genes in the same species. This lack of precise and rigid specificity is well detailed in immunochemical studies (Landsteiner 1947) and frequently referred to by blood group writers.

When an antibody preparation is mixed with a suspension of red blood cells the molecules of antibody preferentially combine with the antigen molecules with which they correspond. When the two do not correspond no combination occurs and the red cells remain free of the antibody. Once the red cells have combined with antibody there are many ways of showing that the union has taken place. Broadly the haemolytic tests exploit the ability of the red cells to dissolve and release the haemoglobin into solution. The surface properties of the red cells may be so altered that they come together in visible clumps; these are the agglutination, antiglobulin, conglutination and enzyme treatment tests.

The choice of technique

It cannot be too strongly emphasised that there are no standard or routine tests which can be simply applied in all species. Even in the same species antibody preparations may require different treatment, and the red blood cells of different species often require different techniques. For example in pigs some antibodies will haemolyse red cells while others, believed to be of the same specificity, will only agglutinate.

Some cattle possess red cells which can be haemolysed or agglutinated with equal amounts of antibody, but other cattle have red cells which can be agglutinated only with powerful antisera. Many of the early attempts to find in the animal kingdom blood groups after the human pattern failed because the technique used in one species with success was so insensitive in another as to be misleading.

Reagents

From these antisera, preparations of antibody are isolated and subjected to further examination. Different serological techniques are compared and the most sensitive and suitable selected. As far as practicable the preparation will be free from antibodies of other specificities. The reactions with red blood cells must be clear-cut and reliable before the preparation is called a reagent and is used as a testing fluid for the detection of the antigen. In cattle attention is also directed to the identity of the newly isolated antibody. By the regular exchange of blood samples between laboratories a close and highly successful correlation of like named reagents is achieved. In the light of the immunochemical evidence however it would be wise to withhold judgement on whether many reagents can be perfectly replicated.

The genetic background

These blood characters have a well defined hereditary nature. The physical basis of heredity lies in the genes which reproduce themselves thousands of times without alteration and are transmitted from generation to generation. The genes are arranged in strings called chromosomes of which each of our domestic animals has two sets, one received from each parent. The gene which controls a blood character, for example, has a definite place or locus on one chromosome. This gene, however, exists in alternative forms called allelomorphs, each of which will modify the red cell antigen in a different and recognisable way. One individual has a pair of allelomorphs which occupy exactly equivalent loci on a pair of chromosomes. If these two genes are the same the animal is said to be homozygous; if different, heterozygous, for this particular character. The genotype is a description of both alleles at a pair of loci. Often, however, the effect of one allele will overshadow or dominate the other and the genotype cannot be directly recognised. Genes whose loci are on the same chromosome are said to be linked. The closer they are together the less frequently do they become separated or recombine as they are passed down the generations.

Blood groups of the dog

As experimental animals for the investigation of problems of blood transfusion and haemolytic disease of the new born in man, the blood groups of dogs have been carefully studied by Young and his colleagues. Five antigens A, B, C, D, and E are reported and several varieties of antigen A are known. The occurrence of each of these antigens appears to be controlled by a single gene whose effect is dominant in the heterozygous condition. No close linkage or allelomorphic relationship has been noticed. Anti D has been found in the serum of normal dogs but it is weak. In the United States 66 per cent. of dogs are A positive and 33 per cent. are A negative. The product, 20 per cent., means that on one in five occasions of average random choice will blood from an A positive donor be transfused into an A negative recipient. If one or two transfusions are given each week for two weeks, 40 per cent. of these recipients may develop anti A during that time, and 80 to 90 per cent. may develop the antibody within six weeks if the injections are continued. This antibody is a potent *in vivo* haemolysin and can cause a serious haemolytic reaction. Other antibodies which may be pro-

duced are much less dangerous in this respect, but they may so shorten the life of the injected red blood cells that the value of the transfusions will be diminished. A second danger lies in using an A positive donor for an A negative bitch and subsequently mating her with an A positive sire. The antibodies in her serum cannot pass the placenta but can be transmitted to the newly born pups through the milk. During the first twenty four hours of life these antibodies can pass through the pup's gastric and intestinal mucosae and cause a haemolytic anaemia of varying severity among those pups with A positive red cells. It seems that natural isoimmunisation of the bitch during pregnacy is rare but the disease could occur as a result of indiscriminate transfusion therapy. Young and his colleagues are of the opinion that these dangers of transfusion will be greatly reduced if A negative dogs are identified and kept as prospective donors. (Young *et al* 1950).

Blood groups of the cat

By careful techniques Holmes (1953) has shown that about 3 per cent. of cats in Britain have sera which react with the red cells of other cats. From these sera two antibodies have been isolated which detect the presence of the antigens E and F. In 103 cats tested antigen F occurred once only. Eyquem has reported finding four different antibodies in France, but it is not known if these include or are additional to those found in Britain.

Blood groups of the horse

The blood of horses can be classified into four groups like the pattern of the human ABO groups. Two agglutinating antibodies are found in normal horse sera which show that horses have one or other, both or neither of two antigens. The antibodies regularly occur in blood from which the corresponding antigen is absent. (Balakrishnan and Yeravdekar 1950; Bruner and Doll 1952). Although this pattern is like that of man the antibodies do not react with human A and B cells, nor do human anti A and anti B specifically react with horse red cells of this blood group system. The saliva of some horses contains substances with A and B specificity. Antibodies specifically combining with human A and B red cells can also be found in horse sera. These serologically active substances do not belong to the blood group system described above. This useful distinction and clarification is by Adams (1958).

It is probable that by some obscure means antigens of the fœtus can get into the mare's system. If she lacks these particular antigens she may form antibodies to them. These antibodies do not affect the foal until after birth when they can be ingested in the colostrum and milk, and a haemolytic anaemia ensues. In the mule breeding areas of France the incidence of the disease is about 8 per cent. of mares at risk and in Thoroughbreds at Newmarket about 1 per cent. The difference is presumably due to the relatively greater antigenic difference for the mare between fœtal antigens of donkey origin and those of horse (Fulton Roberts, 1957). Nine or ten different equine antigens have been found by Franks and other workers (cited in Joysey 1959).

Blood groups of the pig

In Britain a large proportion of pigs carry immune antibodies to the red blood cells of other pigs. This is a consequence of the use of the pooled pig blood used in the preparation of crystal violet vaccine against swine fever. Several potent red cell antigens exist in pigs and many recipients of the vaccine become immunised against them. As a result, haemolytic disease of the baby pig occurs, though not on the scale which might be expected (Fulton Roberts, 1957). Joysey *et al* (1959) have recently identified eleven antigens on the red cells of pigs. Pig blood groups are also being used by Danish workers to assess the progress of inbreeding programmes and to compare the actual rate of increase in homozygosity with the theoretical expectation.

Blood groups of the fowl

Poultry breeders make much use of stocks of birds which have been closely inbred for many generations. In this way they produce less variable and more productive lines. Geneticists, searching for a tool which would disclose some of the hidden consequences of prolonged inbreeding, have adopted blood grouping methods with great success. Immune antisera are prepared by using donors and recipients from different inbred lines. By the use of agglutination techniques seven independent (not apparently linked) loci have been described, several of which are represented by multiple alleles.

It was found by Schultz and Briles in the United States and by Gilmour in Britain, working with different breeds, that even after much inbreeding, when it was expected that all blood group differences would have been bred out, many birds were still heterozygous for several of these antigen genes. It is now known that heterozygous sires are superior in fertilisation rate, heterozygous dams in egg production and heterozygous progeny in liveability. When the parent stocks are being selected, birds of this genetic constitution are more likely to be chosen. It is not known why heterozygosity at these loci should confer these advantages. Big poultry firms find it commercially advantageous to use these techniques to assist the selection of parent stocks. (Gilmour, 1959).

Blood groups of sheep

Haemolytic and agglutination techniques will demonstrate several different antigens in the blood of sheep. Some of the antibodies which detect these were found in the sera of cattle and sheep which have not been injected with sheep blood. Others have been isolated from antisera induced in sheep, goats and rabbits by the injection of sheep red blood cells. Eyquem *et al* (1956) report the presence of ten antigens in sheep with different breed frequencies, some of which also occur in goats. Rasmussen's X and Z antigens form a simple system controlled by two allelomorphs. The R system, however, is complex. Anti R is the most frequently occurring natural antibody and

detects an antigen of the plasma and the red blood cells of R positive sheep. R negative sheep have the antigen r which is detected with a rare antibody of cattle serum. There is also a recessive gene i at a different locus which, when present in the homozygous state, is thought to prevent the formation of the antigens R and r (Rendel, 1957).

The potassium concentration of the red blood cells of some sheep is high with about 36, and of others low with about 11 milli equivalents per litre. Mating data indicate that sheep in the high potassium group are homozygous for a simple recessive gene.

Two forms of the haemoglobin of adult sheep are determined by two allelomorphic genes (Evans *et al*, 1956). Electrophoretically different forms of the serum beta globulins of sheep have also been reported by Ashton (1957).

Blood groups in cattle

The only extensive application of blood grouping in domestic animals is in cattle. Almost all our present knowledge of this subject comes from students of the famous school of immunogenetics built up in Wisconsin by Professor M. R. Irwin. There are eleven blood group systems, the genes for each of which are believed to be on different chromosomes. The D L M Z and Z′ systems are each controlled by two alleles of which one is apparently antigenically inert, and the other controls the presence of the antigen. The FV system has two genes and the antigenic product of each can be recognised; all cattle have one or both of these antigens; no cattle are found to lack both, that is cattle are F+V+, F+V−, F−V+, but never F−V−. The AH system has three genes A, H and an inert form. The SU system has at least four genes of which two are sub-groups and cannot occur independently of the others. The C system has at least eight, some of which are sub-groups. The B system contains well over twenty different genes, several of which occur as sub-groups. In spite of this great variety of antigens in the B system, the red blood cells of some cattle lack every one; these are under the control of the homozygous genes bb. These different systems present serological and genetical features of great technical interest. The J system is the only one to be regularly associated with a naturally occurring, though fluctuating antibody (Stone, 1956). The antigens of this system, of which Oc has recently been discovered (Sprague, 1958) are primarily present in solution in the plasma; only secondarily do the red cells absorb the antigen and become reactive to the antibody. The genetic control of this feature requires clarifying. The genetic control of each of the other blood group antigens is inherited as a simple mendelian factor which is dominant in the heterozygote. This is established by the study of individual families and by large scale statistical investigations. The following data are from Irwin (1956). It will be seen that calves do not possess the antigen when their parents lack it.

Type of Mating	Calves	
	Having A	Lacking A
A×A	1124	141
A×—	603	404
—×—	0	345

At an early stage in the development of the subject it was found that certain antigens occur together more frequently than would be expected by chance alone. For example, when related animals were tested with reagents for B G and K the following results were obtained (Irwin, 1956).

Parents Type of Mating	Numbers of offspring	
	With BGK	Without BGK
BGK×BGK	151	44
BGK×—	185	137
—×—	0	760

With the B system over 100 such genetic associations have been found and confirmed. (Stormont, 1958; Neimann-Sorensen, 1958; Rendel, 1958; Bouw, 1958; Braend, 1959). These compounds are called the B alleles and they are passed from generation to generation as a unit; the individual serological reactions by which they are manifested are regarded more in the light of being facets of different crystals than independent entities.

Any one animal, however, posesses two of these B alleles, one inherited from each parent. It is helpful to identify the alleles present in any animal because they exist in such profusion that they may give a valuable indication of genetic constitution. For example, many breeds have characteristic B alleles. They also give promise of identifying the calves of a bull in a positive way. (Stormont, 1958).

The blood of cattle can also be classified into distinct groups on the types of serum protein and haemoglobin present. On paper electrophoresis, two forms of the adult ox haemoglobin can be distinguished; the faster migrating form is found in Britain only in Channel Island and South Devon breeds (Bangham, 1957). Ashton (1957) claims that three genes, A, D, and E control the bovine serum beta globulins. Any one animal can possess two of these. Genotypically, therefore, cattle can be AA, DD, EE, AD, AE, or DE. Each of these six types of serum can be recognised by its characteristic electrophoretic pattern. It is to be hoped that this technique will become available on a wide scale.

The application of blood group techniques to blood transfusion in cattle

Blood group workers rarely see a clinical reaction in response to the initial intravenous injection of blood. This may be because the volume given is usually less than 50 ml. Larger volumes might be attended by clinical signs of distress. There is no simple test which can be recommended to predict the relative value of different donors for a particular recipient. A donor might as well be chosen for convenience. However uneventful the initial transfusion may be, a second transfusion from the same or different donor given a week or more after, has elements of danger and should be conducted with caution. The blood must be injected slowly and a careful watch kept for clinical signs of uneasiness in the recipient. An anxious expression and a short dry cough are the first signs of distress and may be confirmed by arching of the back, micturition and defaecation. Continuation of the transfusion puts the recipient in increasing danger of

collapse, and, as the injected red blood cells are presumably being rapidly removed from the circulation, the value of continuing the transfusion is entirely negative. Recent work in cattle and sheep using radio active tracers on the injected red cells has shown that new serological techniques are required to assist in the rational choice of suitable donor blood for particular recipients (Bangham, 1956; Drury and Tucker, 1958).

Pedigree registration control and the clarification of doubtful parentage

These facilities depend on the fact that the laws of inheritance are known for these blood group characters and errors in statements of pedigree can be exposed, by the results of examination of blood samples from the animals concerned. The blood group antigens possessed by an individual must also be demonstrable in one or both of its parents. When this is so, the stated parentage may be correct. When the antigen is absent from the blood of the animals stated to be the parents, an error must be present in the records. The aim of the usual investigation is to assist in identifying the sire of a calf from among those bulls by which the dam was inseminated. If all but one are excluded as possible sires, this one is regarded as being the actual sire. When blood from all the animals concerned is examined, solutions can be given in about three-quarters of the cases undertaken. Where the dam has been slaughtered or is otherwise unavailable for test, the chance of excluding the bulls without reference to her blood is reduced to about half of this value (Rendel, 1958). This may, however, be increased with further research.

The occurrence of parentage cases will depend on three factors; first, whether cows returning to heat will be served by the same or different bulls; second, the interval between the services; third, the variation in length of the gestation period; this is influenced by breed, age of dam, season of the year, sex and sire of the calf. According to Rendel, where the service interval is less than fifteen days, there is no certainty of predicting the paternity from the expected calving dates.

Blood group data of bulls, for example, can be recorded and used by scientifically minded persons to clarify questions of parentage. Where any dispute arises, however, the responsibility for a definitive statement must rest with the person who made the initial laboratory tests. On these occasions blood samples from the animals concerned will be re-examined simultaneously in the laboratory under identical conditions, and using special techniques to detect erythrocyte mosaicism.

Blood group methods are increasingly applied to the study of twin cattle. First to distinguish two egg from one egg twins; second, to confirm the clinical diagnosis that a heifer twin to a bull is or is not a freemartin. One egg twins have no genetic differences and are widely used for genetic aand physiological research. They are identified by the failure to find significant differences in characters which are known to be influenced by heredity. Blood group methods are objective, additional to and independent of these morphological examinations. If the members of a twin pair are found to differ in one or more blood group antigens, they are undoubtedly of two egg origin.

About ninety per cent of heifers born twin to a bull have malformed genitalia which render them sterile. They are called freemartins. The bulls are not so affected and are potentially fertile. The anatomical basis of this condition is a junction between the blood circulations of the two developing fœtuses. This anastomosis allows some of the precursors of erythrocytes to wander from one twin cell to its co-twin where they may settle down and produce red blood cells. These red cells, however, must carry antigens of the type with which the precursor cell was genetically endowed. As a result, each member of such a twin pair has red cells of two types which can be distinguished by the different antigens they have. One type of red cells is its own and the other was acquired from its co-twin. This is called erythrocyte mosaicism. This mixture persists throughout life and the relative proportions seem to remain constant. The proportions are not the same in all pairs of twins, however; they are sometimes equal but usually the red blood cells of one type are in greater numbers than the other. When this disproportion exceeds about 90 per cent. to 10 per cent. it is not easily noticed in routine tests, and causes errors in blood typing.

Freemartin diagnosis

This condition can usually be diagnosed after a clinical examination. Where no abnormality can be found, and the veterinarian would like laboratory support for his opinion, this would consist in showing that the blood samples of bull and heifer gave no evidence of mosaicism.

Blood groups and association with disease

It is probable that haemolytic disease of the newborn does not occur in cattle save in an unexpected form. As yet, associations between blood group factors and specified diseases have not been unequivocally established in cattle. In man, such associations have been clearly established (Fraser Roberts, 1959).

Blood groups and association with production characters

There is no convincing evidence that the adoption of blood group methods would offer any help in selecting cattle for improved performance or ability. There is a positive correlation between blood antigen K and high butter fat. This is not causal or dependant but entirely accidental. It results from the fact that both characters are attributes of the Guernsey breed (Stormont, 1959).

Critical, new, and improved statistical procedures for investigating these important possibilities are, however, being explored particularly in Europe, and no doubt elsewhere.

The existence of linkage between the gene for some blood group character and the gene for some other factor such as red coat in Friesian or horned in the polled Ayrshire is also being currently investigated. If

and when found, this will prove invaluable in identifying the carrier calves from particular bulls.

Conclusion

The existence of this huge variety of blood groups suggests to the biologists that it has a selective significance.

"Why are there blood groups?" remains a challenging question.

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References

ADAMS, D. J. (1958). *J. Comp. Path. & Ther.*, **68** (242-252).
ASHTON, G. C. (1957). *Nature*, **180** (917).
——. (1958). *Ibid.*, **181** (849).
BALAKRISHNAN, C. S., and YERAVDEKAR, S. N. (1950). *Indian vet. J.*, **26** (355-363).
BANGHAM, A. D. (1957). *Nature*, **179** (467-468).
——. (1956). Personal communication.
BRAENDT, M. (1959). Blood typing studies of Norwegian cattle breeds. *Thesis.* University of Oslo.
BRUNER, D. W., and DOLL, E. R. (1953). *Cornell Vet.*, **43** (217-222).
BOUW, J. (1958). Blood group studies in Dutch cattle breeds. *Thesis.* University of Utrecht.
DRURY, A. N., and TUCKER, E. M. (1958). *J. Immunol.*, **1** (204-216).
EVANS, J. V., KING, J. W. B., COHEN, B. L., HARRIS, H., WARREN, F. L. (1956). *Nature*, **178** (849-850).
EYQUEN, A., PODLIACHOUK, L., MILLOT, P. (1956). In 15.
FERGUSON, L. C. (1955). The blood groups of animals. *Advances in Veterinary Science*, **2**. Academic Press Inc., New York.
GILMOUR, D. G. (1959). *Genetics*, **44** (14-33).
HOLMES, R. (1953). *J. Exper. Biol.*, **30** (350-357).
IRWIN, M. R. (1956). Blood grouping and its utilization in animal breeding. *VII International Congress of Animal Husbandry, Madrid.*
JOYSEY, V. C., GOODWIN, R. F. W., COOMBS, R. R. A. (1959). *J. Comp. Path. & Ther.*, **69** (29) cited in 17.
JOYSEY, V. C. (1959). Animal and human blood groups. *British Medical Bulletin* **15**, no. 2. The Medical Department, The British Council, London.
LANDSTEINER, K. (1947). The specificity of serological reactions. Revised edition. Harvard University Press.
RASMUSSEN, B. A. (1958). *Genetics*, **43** (814-821).
RENDEL, J. (1957). *Acta Agr. Scand.* VII. **2** (224-259).
——. (1958a). *Ibid.*, 131-161.
——. (1958b). *Ibid.*, 162-190.
——. (1958c). *Ibid.*, 191-215.
ROBERTS, J. A. FRASER (1959). Blood Groups and Disease. *British Medical Bulletin*, **15**, no. 2. The Medical Department, The British Council.
ROBERTS, G. FULTON (1957). Comparative aspects of haemolytic disease of the new born. Heinemann, London.
SORENSEN, A. NEIMANN- (1958). Blood groups of cattle. Mortensen, Copenhagen.
SPRAGUE, L. M. (1958). *Genetics*, **43** (906-912).
STONE, W. H. (1956). *J. Immunol.*, **77** (369-376).
STORMONT, C. (1958). On the applications of blood groups in animal breeding. Proceedings of the X International Congress of Genetics, Montreal.
YOUNG, L. E., O'BRIEN, W. A., SWISHER, S. N., MILLER, G., YUILE, C. L. (1950). *Amer. J. Vet. Res.*, **13** (207-213).

THE SPEAKER'S INTRODUCTION

The Chairman, Dr. K. C. Sellers, introducing the author, said that Dr. Hall was one of the few veterinary surgeons in this country who was working on blood groups. This subject had been rather neglected, and he felt sure that Dr. Hall would indicate the various implications involved and its great value in our domestic animal economy. He invited Dr. Hall to present his paper.

Dr. J. G. Hall, in presenting his paper, said that essentially blood-grouping consisted of the recognition of clear-cut individual differences in the blood of a single species. Conventionally, these differences were based on the presence or absence of a hereditary erythrocyte antigen. A wide variety of these could be found in most domesticated species. Similar clear cut differences between individual animals were exhibited by other blood constituents such as haemoglobin and serum proteins. He ventured to prophesy that blood group workers would pay increasing attention to the recognition of such biochemical variants and their genetic control.

The subject could be approached from several points of view according to personal preference. New knowledge was needed at every point, however, and a great deal of hard and exciting work lay ahead for everyone in the field. The main applications of blood group methods were in the prevention and diagnosis of haemolytic disease of the new-born in the pig and horse; in the control of pedigree registration of cattle; and in the understanding and manipulating of the genetic constitution of different breeds. In the poultry industry the blood groups of birds were taken into account when parent stocks were being selected.

He felt sure that blood transfusion therapy would be more widely used, particularly in dog and cattle practices, if clinicians could more easily assess the dangers. This was a subject which could be most profitably investigated; using, perhaps, radioactive techniques. He thought we should make intelligent use of the principles of medical experience in this field, though it would be unwise to use the same serological techniques.

The Opener

Dr. R. F. W. Goodwin, opening the discussion, said that there were two main reasons why he was pleased to be associated with Dr. Hall's paper. First, Dr. Hall and he had worked in the same building several years ago and had shared the same technician. Since those days Dr. Hall had devoted himself wholeheartedly to the study of cattle blood groups, and it was a personal pleasure to see his patient work recognised at this Congress. It was an excellent thing that increasing numbers of veterinarians were entering research fields that were regarded until quite recently as being outside their professional province. Any mechanism, such as these annual Congresses, whereby they could return to the fold to talk about their work must give them encouragement and benefit us all.

The second reason why he was glad to be present was that the subject was an important one. It was an important one in its own right, as could be seen from the paper itself, and also because of its bearing on veterinary science. Subjects such as this had implications extending beyond the day-to-day work of veterinary practice. He congratulated the organising committee, therefore, on their choice of this and several other papers this year, which underlined the expanding interests of veterinary research workers.

They were all indebted to Dr. Hall for summarising very clearly and simply a subject that was so littered with the jargon of the specialist that it could readily be made incoherent to the general reader. Following his good example, he would confine his remarks to broad questions rather than to points of technical detail. He thought that if at the beginning they kept on a broad basis they could cover the many ways in which blood groups touched upon them as a profession, and then perhaps, later, people could pick out more specialist and technical points and they would do their best to answer questions.

As Dr. Hall had said, the blood groups of animals concerned them as veterinary surgeons at three quite different levels. First, they impinged on veterinary practice in its day-to-day routine, as in blood transfusion and haemolytic disease. Secondly, rather more removed from day-to-day work in practice, they were tools for the animal breeder and livestock improver. Thirdly, and perhaps even further away, they might help to provide the answer to wider questions of medicine, especially as these affected man. He intended to examine each of these categories in turn and enlarge upon them.

In the treatment of disease there had been an increasing appreciation of the value of nursing and general supportive therapy. This was particularly so in the surgery of the dog and the cat, where in the last few years the standards of anaesthesia and surgical technique had advanced so rapidly

that deaths from fluid imbalance now figured more prominently. As anaesthesia became more reliable the things from which they saw animals dying many years ago were now very minor risks, and their attentions were forced to more general questions that they had not faced up to before, such as what was killing an animal in the way of shock and that sort of thing. His surgical colleagues at Cambridge made much use of blood transfusion, and would make considerably more use of it were the knowledge and facilities available for running a more extensive blood bank.

In surgery, the prime use of blood transfusion was to counter the effects of acute haemorrhage and anaemia. It had an important post-operative therapeutic role, therefore, but it was also used by his colleagues to improve operative risks. Thus all dogs with a haemoglobin level of less than 7g/100 ml. were now routinely transfused before surgical operation was undertaken. Another indication for blood transfusion was in shocked obstetrical cases. They had quite a number of cases where the animal came to them in a rather shocked condition, and transfusion improved the animal's chances.

Those present on Tuesday afternoon might have been surprised that so many practitioners were getting on well in this field. There was an extraordinary degree of interest and thirst for knowledge. The subjects touched upon were parasitism and the dominating role of anaemia in diseases of that type. One speaker mentioned Redwater in cattle and said that they had transfused about 50 cows, with a great deal of success. Just as improved techniques in surgery forced them to look more at what was really going on in the animal, so with improved anthelmintics they would have to pay more attention to the basic questions of supportive therapy.

Haemolytic disease of the new-born was a problem in the horse and the pig. In neither species was the disease of major economic importance, but it contributed to the heavy mortality of the neonatal period. The management of the condition differed greatly. In the horse, the recognition of the potential danger before term, from the level and specificity of the maternal iso-antibodies, allowed preventive measures that were considerably more rewarding than the exchange-transfusions of already affected foals employed previously. In the pig, the offspring had not the same economic importance, and it was not practicable to test sows for high antibody titres. Neither was it such a simple matter to feed the new-born artificially for two days, as with foals, until the gut became impermeable to the haemolytic antibodies. Once a sow lost a litter with haemolytic disease, she was best slaughtered, as it was very common for successive litters to be similarly affected.

They, at Cambridge, had been very interested in this question of haemolytic disease in the pig. They knew that nearly all, if not all, the cases of haemolytic disease in the pig in Britain had been induced by injections of crystal violet vaccine, and from the beginning it was both striking and puzzling that the disease did not seem to occur in pedigree Large White litters. When he said that the disease did not occur he really meant that clinical or fatal cases did not occur. They all knew that the widespread use of the vaccine induced some antibody response in a high proportion of the animals that had been vaccinated twice or more, and mild disease (as evidenced by the presence of antibody on the neonatal red cells) was common. This absence of the disease in Large Whites could not have been foreseen, and it was indeed fortunate, for the Large White breed had provided the major portion of the sow population in the past.

It was likewise fortunate that the recent big increase in the number of Landrace pigs in this country would not alter the situation, as the Landrace breed appeared to be almost equally immune. This immunity of the white breeds was intriguing, and they had tackled the problem repeatedly. Having isolated 11 blood-group antigens in the pig, they recently studied their phenotype frequencies in seven different pedigree breeds. This work had confirmed that the white breeds were remarkably homogeneous for the three main antigens that, so far, had been incriminated in their cases of haemolytic disease. This meant that the chances of incompatible matings were slight, particularly as one of these three antigens did not occur among Large Whites; so that even if the corresponding antibody was widely stimulated there were no boars to provide the antigen on the red cells of the new-born. Nevertheless, they would still have expected some cases of haemolytic disease among the very large population of vaccinated white sows and, as a result, they had recently tried to produce the disease by vaccinating a number of pedigree Large White sows experimentally. The disease had been produced without very great difficulty by this means, and hence further examination of this question would be necessary. So much for the day-to-day work on the pig.

In the field of breeding and livestock improvement the horizons for blood-group work were wider and, although more distant, they were lands of promise. As others better qualified than he continued to point out, large numbers of animals in Britain that they as a profession tried to keep healthy were not well designed for their function in the first place. The scope for livestock improvement was still very great, and there was as much to be gained in breeding potentially better stock, as in improving the environment of the existing animal population.

Dr. Hall had emphasised the manner in which blood groups were a measurable expression of a basic genetic pattern. Why was one animal better in performance than another? What had it that made it superior? When they could relate those questions to blood-group patterns they might bring some definition to a previously hazy subject. The work of Briles in this respect with poultry, and also that by Gilmour in Cambridge, had attracted world-wide interest. There were indications that certain blood-group patterns in poultry were associated with better laying ability, and the commercial people had lost no time in employing blood-group techniques. Perhaps not in this country, but in Canada, this work of Briles had been the stimulus for setting up blood-grouping laboratories. The Swedes and Danes were getting on very fast with the blood-grouping of pigs, and the Americans were pressing ahead also.

Dr. Hall had given particular attention to the use of blood groups in cattle-breeding programmes, particularly as an aid to identification. Many practitioners would agree that identification of animals had become quite a problem in many ways, even in simple matters on the farm, as in tuberculin testing and vaccination, and they would appreciate the importance of blood tests such as these. The growth of artificial insemination increased the need for some means of labelling the offspring, and it would probably not be long before they witnessed an important expansion in the number of pigs born following artificial insemination. The registration of livestock births was so patently dependent on goodwill that it would be useful to have tests that, even though they might not always provide the answers, could often detect fraud or clerical error.

While touching upon this wider question of genetic constitution, mention could be made of the relationship between blood groups and susceptibility to disease in man. Persons of blood-group A were more susceptible to carcinoma of the stomach and some other diseases, while women of the same group more commonly suffered from genital carcinoma. Duodenal and gastric ulcers occurred more frequently in people of Group O, and these observations had caused many to wonder whether they were measuring some generalised factor in constitution that accounted for the long-observed differences in disease susceptibility between different people. For the moment, however, it was safer to assume that these diseases, which were mostly of the digestive organs, were more specifically related to the presence or absence of blood-group substances in the affected organs themselves.

On the third main category, that of comparative medicine, little need be said. They were now reaching a position where research carried out on animals for its own sake was contributing to the sum of general medical and biological knowledge. Many questions applicable to disease in man might be answered by experimental work on animals. Confining this argument to haemolytic disease alone, there appeared to be a major difference in the mode of sensitisation between the two animal species affected with haemolytic disease. In the mare, the stimulation for antibody production appeared to be a natural phenomenon, as in man; yet in the pig, which was very similar in some aspects of placentation, the natural disease might never occur. The natural route of antigenic stimulation in women had long been a puzzling

subject, and differences such as these might help to elucidate the problem. Yet again, in their work on the disease in pigs, his medical associates at Cambridge had been interested in their observation that sows fell into two groups according to the duration of their iso-antibody response. In many animals the iso-antibody response was short-lived, but in others the antibody titre persisted unchanged and at a high level for several years, despite the absence of any further obvious antigenic stimulation, and also in the absence of pregnancy. This had very interesting implications.

Comparisons such as these could be easily continued and also extended into other fields of blood-group research, and they underlined the rapidly growing inter-relationships between veterinary and medical knowledge.

The General Discussion

Mr. G. F. Smith (M.M.B., Thames Ditton): "The Milk Marketing Board's interest in blood grouping was aroused some 2 years ago when it was felt that their work in the field of livestock improvement was sufficiently important to warrant a parentage check, and up to the present time some 200 calves born to become future A.I. sires have had their parentage checked, using the Copenhagen blood grouping service. A little over 2 per cent. error has been recorded.

"Besides the actual practical value of blood grouping we must, I feel, also realise that in the veterinary field we still have much to learn from the bloods of our domestic animals, and future research and extra techniques may show that factors of major economic importance may be located in this field; in fact, factors may come to light which help to pinpoint yield of milk and fat, disease resistance and fertility and, therefore, the more effort that can be put into this whole research field, the better for the livestock industry as a whole."

Mr. A. L. Ogden (A.H.T., Stock, Essex): "I have read Dr. Hall's paper with great interest and should like to congratulate him on the concise and characteristically thorough way he has dealt with a most difficult and involved subject. He stresses the immediate practical applications—transfusions, parentage testing, free-martin diagnosis—but I feel he has not stressed enough what, to me, is the more important aspect of his subject, namely the genetic implications of this and other allied techniques. There is a wealth of evidence, though much of it is at present made on lesser species, such as snails, that polymorphism (that is the existence of two or more discontinuous varieties in the same species at the same time in such proportions that the rarest of them cannot be maintained by recurrent mutation alone) constitutes the raw material of adaptation and evolution. One of the dimorphisms that Dr. Hall has mentioned, that of sheep haemoglobins, is an example. Two distinct varieties of haemoglobin in the same species and under genetic control, with one variety (Haemoglobin A) possessing a much greater affinity for oxygen than the other form produced in response to its allele. This has led to selection for the allele controlling haemoglobin A in mountain districts, where the oxygen content of the atmosphere is low, so that the frequency of this gene in breeds indigenous to such environments reaches 0·8 Lowland breeds on the other hand in environments where atmospheric oxygen is adequate, find B type haemoglobin more satisfactory—its lower affinity for oxygen allows it to release oxygen to the tissues more easily and it is this allele that is found at high frequency, around 0.8. This provides a clear cut example of the adaptive value of these biochemical variants and of the reason why biologists are interested in these genetically different types.

"Polymorphism has been described as a means of storing up energy against environmental change. It is a species survival mechanism and in some cases, as in the sheep example, extends the range of land types colonisable by the same species. It is highly probable that the important problem of heat tolerance in cattle will yield to this type of biological and genetic analysis.

"If the blood groups of animals *are* another example of a balanced polymorphic system—human blood groups have been treated as such since 1945—then the frequencies of the various types can be expected to be held in equilibrium by a balance of selective forces, the outcome of the advantages and disadvantages associated with each particular type. In other systems—including the human blood groups—differential disease susceptibilities, fertilities and viabilities, slight differences in sex ratios as well as physiological differences of the sheep haemoglobin type have all been shown to play a part in maintaining the diversity of types in balanced equilibrium. The demonstration of these differences has only been possible by the very widespread routine typing such as that now practised by hospitals in the case of patients suffering from certain diseases or that applied at maternity and antenatal clinics. It is difficult to see how in the absence of some equivalent blood testing service the necessary population data can be obtained to permit comparable studies to be made on farm animals.

"I should very much like to hear Dr. Hall express his views on this aspect of blood grouping; to hear whether he considers the veterinary profession might also be concentrating its blood typing facilities on certain specific diseases; whether these services need extending, and whether some at least of the genetic markers now available, including the blood group antigens, could be incorporated with advantage in any of the disease incidence surveys so popular in the veterinary field today."

Dr. W. R. Wooldridge (London): "I must rise in an effort to dispel some of the pessimism expressed by Dr. Holman.

"We must remember that this work of blood grouping in domestic animals is still in the early stages of development. In our work at the Equine Research Station of the Animal Health Trust we have had considerable experience now in the transfusion of blood but in the early days we did get untoward effects when blood of the wrong group was inadvertently used for transfusion. Now we take considerable care to ensure that our "blood banks" are of the right type. Whilst we know that there are blood groups for horses it is, however, necessary to work the matter out more fully.

"Mention has been made of the desirability of taking note of other characteristics and one might mention here the work of Ashton at our Farm Livestock Research Centre whereby he has examined serum protein strains in a large number of cattle, sheep and pigs. Ashton has used a comparatively simple technique of electrophoresis, using a starch gel and separating the serum protein on this gel. By this technique he has found, for instance, that the many hundreds of cattle he has examined all fall into 6 protein patterns. He has shown that these patterns are hereditary and efforts are being made to link them with economic factors. Distinctive patterns have also been found for the serum proteins of sheep and pigs.

"I personally feel that this work is exceedingly important and is likely to prove even more so when we have further information available to us and there is no doubt that we owe a debt of gratitude to people such as Dr. Hall and Dr. Goodwill who have devoted themselves to this pioneer work."

Dr. K. C. Sellers (A.H.T., Stock, Essex): "Before concluding the discussion, I should like to say a few words and ask Dr. Hall a question. In his paper he has mentioned the serum Beta-globulins and Starch-gel electrophoresis. As Dr. Wooldridge has indicated, this technique is being used at our Research Laboratories at present on a semi-routine scale. We hope to be carrying it out as a routine in the New Year.

"In Dr. Hall's paper he has used the term "Blood Groups" as a general title for the various polymorphic systems which are at present being used in human beings and animals as genetic markers, but does he think that "blood groups" is the right term to cover such a wide variety of techniques and should not a more general description be used?"

The Replies

Dr. J. G. Hall, in reply, said that the blood transfusions which he often gave to cattle were experimental and not therapeutic in nature. They had the aim of stimulating antibody formation. The antisera thus formed were the source of the antibody reagents used in the blood grouping tests. At weekly intervals 50 mls. of whole citrated blood were given intravenously through a fine flexible polythene cannula. The initial injection had never been accompanied by signs of distress in the recipient though this had been recorded by others. The second injection, however, elicited an imme-

diate clinical response in about 10 per cent. of recipients. The signs were an expression of anxiety; the respirations became faster and shallower and were nearly always associated with a short, sharp, asthmatic type of cough. Usually the animal would make an uneventful recovery within the hour. In severe reactions, the coughing was continual. Micturition and defaecation took place. The animal appeared to fear falling down and would stand with legs straddled and the back arched, trembling and coughing. He would suggest that blood transfusions must be given cautiously and a sufficiently large amount of blood should be given initially to avoid the necessity for repetition. In cattle, the cross-matching of recipient and donor blood was not regarded as providing a reliable guide to the subsequent *in vivo* behaviour of injected red blood cells. Research was needed at this point.

Recent work using donor red cells labelled with radioactive chromium in man, cattle and sheep had shown that stored blood was removed from the circulation of the recipient more quickly than fresh blood. This might reduce the value of the transfusion. Blood collected at the abbatoir was often highly infected and the danger of transfusing this material would be obvious.

Dr. Hall was glad that the matter had been raised once more of a cattle blood group service for the country. These methods gave indispensable help in identifying the paternity of calves born to dams inseminated by several bulls at close intervals. The necessary reagents and research experience were now available in this country and most questions of pedigree and others, could be solved by the application of immunogenetical principles. So far, however, no one had been found willing to take the responsibility for running a blood group service to which breeders and veterinarians could have easy access. At the present state of knowledge it should not be claimed that a blood group service would give direct help in improving the production characters of beef or dairy cattle. He believed, however, that such a service would produce results of importance to veterinary science as well as providing information for the agricultural community. He hoped that the difficulties would be vigorously tackled and surmounted by the potential users of the blood group service. He gave a warning, however, that the service would inevitably be called to settle disputes. However rare these might be the scientific basis on which decisions were founded must be beyond criticism and the quality of the workers and their facilities must not be inadequate in any sense. He hoped the veterinary profession would be associated with the blood group service.

Dr. Goodwin said that haemolytic disease in pigs was most common when a sow was a black sow. The incidence of the disease was roughly proportionate to the incidence of the black breeds. In the past when there were Essex and Wessex pigs, the disease was most common in the offspring from Essex and Wessex sows. He had also seen it in large Blacks and Berkshires. The reason was that the black breeds of pigs were very mixed in their blood group patterns.

Dr. Hall said that it had been established that in poultry parent stocks of fowls could be selected to produce offspring of certain blood types. These birds produced more eggs and lived longer than those of other blood types. The reason was unknown but the chosen blood groups were presumably reflecting an appropriate genotype. The advantage was commercially attractive for the big breeder.

Finally, he said how much he had enjoyed discussing the range of questions posed. The points which had been put to him had been most helpful.

Dr. R. F. W. Goodwin, in reply, said that he did not have a lot to do with blood transfusions at Cambridge. With dogs, Dr. Hall used a human giving set. With cattle, the system was not used so extensively. There were certain problems about getting the blood and storage. They were in the early stages, and mostly it was used in obstetrical cases where the important thing was to get the patient out of the shocked condition.

While on the question of transfusion, it seemed worth while to point out that all the things that went wrong were not necessarily to do with blood groups. Odd things happened in human blood transfusions and the trouble could be put down to such things as substances that came from the rubber tubing, dirty tubing and glassware and, of course, infective agents in the blood. These peculiar reactions were not necessarily always related to blood-group incompatibility.

There was one last point on a general note that was worth stressing. This was a long-term business. Perhaps those who had not worked with blood groups did not realise that it could not be greatly hurried. They could quicken the pace perhaps, with more people and money, but it was still a long-term business. If they wanted to use blood groups in a species in 20 years' time, they had to start working for it now. They had to lay the foundations for many years ahead, because there were so many groups to be laboriously identified.

The Chairman asked whether the meeting desired the following resolution, submitted by Dr. R. F. Montgomerie, to go to the Annual General Meeting:

"That this sectional meeting of the B.V.A. Congress, conscious of the material research advance made in the study of the blood of animals in relation to blood grouping and associated factors, is satisfied that the time is ripe for a considerable extension of this research and the provision of a service in this subject;

It recommends that the Technical Development Committee be asked to take up the matter as an urgent question with a view to the full establishment of research and service facilities in this field."

Dr. H. H. Holman seconded the resolution and it was carried unanimously.

The Chairman thanked Dr Hall for his excellent paper and for the way in which he and Dr Goodwin had dealt with the discussion.

The Sense of Taste in Domesticated Animals

BY
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SUMMARY—1. The anatomical disposition of the taste receptors (taste buds) and the course of the afferent nerves to the higher centres are briefly described.

2. The results of electrophysiological investigations where the taste receptors are stimulated by chemical solutions and the activity in the afferent nerves recorded are discussed. There are many species differences which can be demonstrated by this technique, although all species react to salt, sour, sweet and bitter solutions.

3. The technique of taste discrimination by two choice preference tests is outlined. This method also shows that the limited number of domesticated animals so far investigated can discriminate between varying concentrations of salt, sour and bitter. The goat and ox take all strengths of glucose solution with avidity.

4. The preference shown for certain substances can be affected by the biochemical needs of the animal. Self-selection of diet probably plays an important part in an animal's normal feeding and this is probably regulated by taste.

5. Species show marked variation in the sense of taste, but in all animals taste probably plays a protective as well as a nutritional role. The primary need for calories, however, may override any endeavour to compensate for mineral or other deficiency.

6. When the biochemical equilibrium of the animal breaks down, then perverted appetite may develop. By habituation the perverted appetite may persist after the biochemical unbalance has been restored.

Introduction

TASTE, or gustation, is a chemical sense which was probably evolved during the aquatic stages of the evolution of vertebrates. In the hierarchy of the senses possessed by the domesticated animals, taste can be regarded as one of the most primitive. Taste continues, however, to subserve the fundamental biological role common to all the senses in that it provides a means for preserving the species by forming a link between the animal and its environment. Through the sense of taste the animal is able to regulate, to some extent, the intake of suitable food and the rejection of unsuitable or poisonous food. By itself the sense of taste is probably not vitally important to the welfare of the animal in the way that sight or hearing is, for an animal deprived of sight or hearing would be immediately at the mercy of its environment.

The sense of taste receives much reinforcement from other senses, especially that of smell, which can be regarded as taste projected to a distance. Touch receptors and thermal receptors also aid taste in assessing the qualities of the ingested food as it is moved around the mouth by the tongue and cheeks. The sense of taste, therefore, forms part of the perceptive processes of an animal by which it gauges the palatibility of any food material. When taste is completely abolished (ageusia), and there is no evidence that this clinical condition ever occurs in animals, the other chemical senses such as smell and the common chemical sense are used to assess the nature and composition of the prehended food.

Because of the control which man has exerted on the environment during the domestication of animals, the protective function of the sense of taste has necessarily been reduced in importance. The housing of animals, together with restriction of grazing, limits the free movement of animals, thus preventing them from making a natural selection of food and causing them to ingest grasses which may predispose to such disease entities as acute tympany. On the other hand, the high productive standards demanded of farm animals today require the ingestion of relatively enormous amounts of food so that taste as a component of "appetite" and "palatability" assumes greater importance. Associated with this provision of sufficient food for the high energy requirements demanded by the highly productive animal there is often a disruption of the mineral balance of the animal's body which becomes manifest as deficiency diseases, e.g., hypomagnesaemia and hypoglycaemia.

This paper discusses the somewhat limited data available on taste in animals. The data have been derived from physiological experiments designed to examine variation in the sense of taste by electrophysiological studies and by preference tests. In addition, some information has been obtained from experiments and observations on animals which may show profound alterations in taste thresholds when changes in the electrolyte balance of the body occur, either by mineral imbalance or hormonal deficiency. Observations of behaviour in which perverted taste is a symptom are discussed in the light of the available experimental information.

Anatomy

The receptor cells for the taste sense are chemoreceptors which consist of spindle-shaped cells grouped together to form a central core surrounded and supported by fusiform cells. Each gustatory cell has a filamentous prolongation directed towards the taste pore of the taste bud which opens on the surface of the buccal epithelium.

Most of the taste buds are situated on the dorsal surface of the tongue and they are most frequently associated with papilliform outgrowth of the lingual epithelium. Taste buds also occur on the palate, and in the pharynx and larynx. Variations in the occurrence of different types of papillae are accompanied

by similar variations in the disposition of the taste buds in different species. For example, the circumvallate papillae in the ox are arranged in two rows of about 10 at the base of the tongue, while in the horse only one relatively large papilla occurs on each side. Other species of ruminants have numerous circumvallate papillae like the ox, while the carnivora have only one to three on each side .The poorly developed tongue of birds is devoid of papillae and apparently of taste buds.

There are very many taste buds associated with each type of papillae; as many as 30,000 have been observed in the ox. The arrangement of this enormous number of receptors on the dorsal surface of the tongue ensures that the presence in the mouth of substances capable of being tasted is conveyed to the brain. Taste buds may be absent in the neonatal animal and in man, the number decreasing with age (Arey, Tremaine and Monzingo, 1935). This may reduce the sense of taste in old people (Richter and Campbell, 1940). Watson (1944) has shown that, in lambs, milk is a more efficient activator of the oesophageal reflex than water, so presumably the suckling lamb has well-developed taste buds. There is no collateral evidence available of senescence of taste buds for the domesticated animals, and because of the short life span of these animals it is doubtful whether any reduced sensitivity would be of biological significance.

The taste buds of the anterior two-thirds of the tongue are innervated by nerve fibres which pass centrally in the chorda tympani, a small branch of the seventh cranial nerve (facial). The taste buds of the posterior third of the tongue send afferent fibres to the brain by the ninth nerve (glosso-pharyngeal). The nerve impulses originating from stimulation of the taste receptors pass first to the sensory nuclei in the medulla oblongata, where they are closely associated with the salivary nuclei and nuclei controlling mastication and tongue movements. From the medulla taste impulses are carried forward in the brain to the thalamus and thence radiate to the orbital pole of the cerebral cortex (Patton, 1950). The nervous pathways for taste pass is close proximity to the other sensory paths for touch and warmth.

Interruption of the sensory pathway for taste alters the taste thresholds, so that unpleasant fluids such as strong acid or concentrated quinine solutions may be imbibed without any sign of aversion (Patton and Ruch, 1944; Andersson and Jewell, 1957).

Experimental Methods of Assessing Taste

Electrophysiological Investigations

The activity of any nerve can be recorded in the anaesthetised animal by dissecting the nerve free, placing it on silver electrodes and displaying the resultant bioelectrical activity on an oscilloscope after suitable amplification. In the case of taste the activity

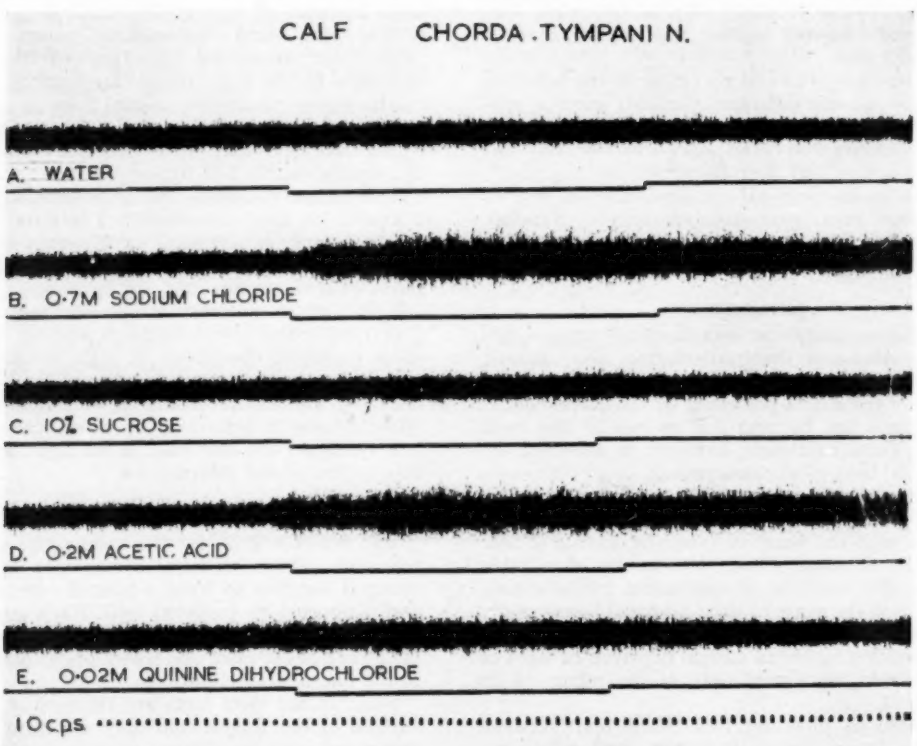


FIG. 1.—Oscillograph records showing the activity of the whole chorda tympani nerve. Time marker shows intervals at 10 per sec. The stimulus to each trace is indicated on the figure and the time of application is indicated by the downward shift of the baseline.

of the chorda tympani and glosso-pharyngeal nerves is recorded when the taste buds are stimulated by various sapid solutions.

Zotterman (1935) demonstrated that stimulation of the chemo-receptors of the tongue of the cat by salt, bitter and acid solutions caused a large discharge of impulses in the gustatory nerves, but sweet solutions produced no such response. Dogs, on the other hand, reacted to sweet solutions (Andersson, Landgren, Olsson and Zotterman, 1950), as do the rat and rabbit (Pfaffmann, 1953), hamster and guinea-pig (Beidler, Fishman and Hardiman, 1955), pig (Zotterman, 1956) and goat, sheep and calf (Baldwin, Bell and Kitchell, 1959). The apparent absence in the cat of fibres showing activity to sugar solutions is surprising, since all other species examined do possess sweet chemo-receptors. The absence of sweet receptors in the cat must be regarded as being somewhat equivocal, for both Pfaffmann (1941) and Liljestrand and Zotterman (1954) agree that "in the cat there are receptors for sweet though probably not very numerous". It is possible that reactions to sugar solutions may be difficult to elicit in the cat because of the very small diameter of the afferent nerves and the scanty disposition of sweet receptors. Baldwin *et al.* (1959) have been able to improve the definite gustatory responses evoked in ruminants by mechanically moving the circumvallate papillae at the same time as the sugar solutions are applied. It is possible that the mechanical movement allows the sugar solution to flood the fossa of the circumvallate papillae and so stimulate the hidden receptors, an action which would normally be brought about by the well-known movements of the tongue in "tasting".

The existence of specific fibres in the frog responding to water was described by Zotterman (1949) and extended by Liljestrand and Zotterman (1954) to the cat, dog and pig, and from their findings they postulate the presence of a "water-taste" in all mammals. More recently Zotterman (1956) has shown the absence of any water-receptor in the rat and Baldwin *et al.* (1959) could not evoke any response when water was applied to the tongue of the goat, sheep or calf.

In addition to the variations mentioned above between species, Pfaffmann (1953; 1955) and Beidler *et al.* (1955) have demonstrated the occurrence of many qualitative differences between species on stimulation with sapid solutions. These electrophysiological results emphasise that any general theory of taste must take into account species differences.

Recent experiments of Cohen, Landgren, Strom and Zotterman (1957) show that the gustatory impulses generated in the mouth are received on the orbital surface of the cerebral cortex. This region corresponds to an area of the cerebral cortex shown by Patton and Amassian (1952) to be activated by the chorda tympani. Bilateral destruction of this region is known to cause impairment of taste in a variety of animals (Benjamin and Pfaffmann, 1955). Bremer (1923) has shown that this region of the brain in the cat is intimately associated with the tongue and masticatory muscles.

Preference Tests

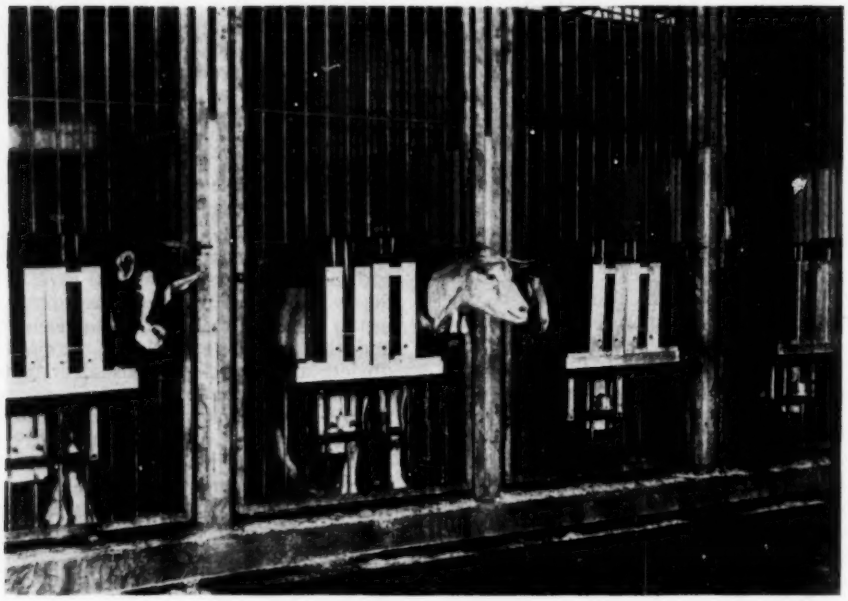
In man, taste thresholds can be evaluated subjectively by the "drop" or "sip" method, but in animals thresholds must be ascertained by discrimination techniques.

Taste discrimination by means of tests whereby an animal is permitted a choice between a sapid solution and water has been investigated in some laboratory animals (see Patton and Ruch, 1944). This type of procedure has been extended to the goat (Bell, 1959) and to cattle (Bell and Williams, 1959), but not so far to other domesticated animals.

For this type of test, fluid is offered from two identical containers, one with the solution to be tested and the other with tap water (Fig. 2). The amount of the two liquids provided, tap water and test substance in tap water, is measured at the same time each day and the amounts consumed recorded. In order to obviate any bias due to visual or behavioural cues, the position of the test solution is reversed at the end of 24 hours. The amount of test solution taken during the 48 hours' test period is expressed as a percentage of the total fluid intake (i.e., test solution plus water). These figures, when plotted against the concentration of test solution, provide a curve which shows the rejection threshold. At high concentration of test substance the amount taken is very small, usually only sufficient for the animal to taste; consequently, the rejection threshold is set arbitrarily at the lowest concentration where not more than 20 per cent. of the total fluid has been consumed. Offering the test solutions in descending or ascending concentration has been shown to be without effect on the overall pattern of the results. As the concentration of the solution is progressively decreased from the rejection threshold, the amount of the test solution imbibed begins to increase, until the animal shows no discrimination between the test solution and water. At this point of no discrimination, between 40 per cent. and 60 per cent. of the total fluid intake is test solution: that is, the animal accepts the test solution as readily as tap water. This point is accepted as the acceptance or non-discrimination threshold.

It is possible, using this type of test, to make comparative measurements of taste thresholds between different species. The results for goats tested against the four taste modalities of bitter, sweet, salt and sour are seen in Fig. 3. There is little other data available for comparison between the domesticated animals because very few experiments have been made. Table I shows the rejection and acceptance thresholds of a number of species for bitter taste and emphasises the marked species difference in gustatory discrimination. The goat has a very high threshold for bitter taste when compared with other species. It is possible that during its evolution the goat, being a browsing animal feeding from shoots or leaves which are normally quite bitter, has become adapted to higher bitter taste thresholds. The marked difference between the threshold of the two ruminant species, goat and ox, is most striking and can possibly be explained by the contrast in grazing behaviour, natural habits and food of these species.

A comparison of the thresholds of the ox and goat is given in Table II. The results using glucose as test solution did not provide data which could be used for the assessment of thresholds (see Fig. 3). Both



A

B

C

FIG. 2.—A. Shows the range of pens used to house the goats for preference tests. The containers for the bottles are inset into the gates, which allows for easy cleaning, replacement and measurement. B. The arrangement whereby test solution and drinking water are provided to the goat. C. The arrangement adopted to allow cattle access to the two fluids. (Fig. 2 A and B are reprinted with permission from Bell, F. R. (1959), *J. Agric. Sci.*, **52**, 125.)

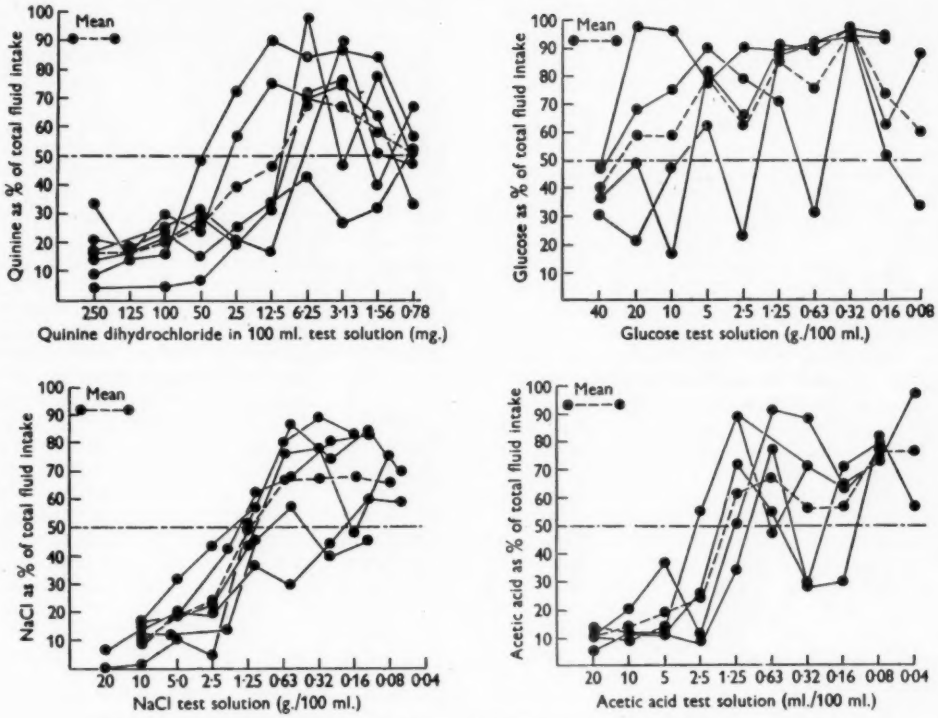

Mean
Quinine as % of total fluid intake
Quinine dihydrochloride in 100 ml. test solution (mg.)
Glucose as % of total fluid intake
Glucose test solution (g./100 ml.)
NaCl as % of total fluid intake
NaCl test solution (g./100 ml.)
Acetic acid as % of total fluid intake
Acetic acid test solution (ml./100 ml.)

FIG. 3.—Shows the results of preference tests for goats against the primary taste modalities of bitter, sweet, salt and sour. (Reprinted with permission from Bell, F. R. (1959), *J. Agric. Sci.*, **52**, 125.)

TABLE I

TASTE THRESHOLDS FOR BITTER TASTE IN VARIOUS SPECIES (QUININE, MGM./100 ML.)

Species	Rejection threshold	Acceptance threshold	Authors
Goat	125·0	12·5	Bell (1959)
Monkey.. ..	25·0	3·1	Patton and Ruch (1944)
Chimpanzee ..	6·2	0·3	Patton and Ruch (1944)
Man	2·5	0·9	Engel (1928), quoted by Patton and Ruch (1944)
Rat	0·3	0·05	Patton and Ruch (1944)
Rat	1·6	0·7	Weddell (1936)
Ox ..	1·9	0·12	Bell and Williams (1959)

goats and cattle accepted the highest concentration of glucose offered, namely 40 g/100 ml., and showed a marked preference for glucose solution over water. In the calves this preference for glucose solution caused a certain amount of scouring, the animals losing condition, following the onset of anorexia. Frings (1946), using preference test techniques, has shown that many animals, including the horse, dog and chicken, prefer sweet solutions to water. In the cat Frings (1951b) showed that diluted milk containing sugar was preferred to diluted milk alone. He believes that a widespread distribution of this preference for sweet solutions is a phenomenon biologically important from the nutritional point of view.

TABLE II

TASTE THRESHOLDS OF GOATS AND CALVES TO THE FOUR TASTE MODALITIES OF BITTER, SOUR, SALT AND SWEET

	Goat Threshold concentration (moles)		Calf Threshold concentration (moles)	
	Rejection	Acceptance	Rejection	Acceptance
Quinine dihydrochloride	0·0031	0·00031	0·000097	0·000024
Acetic acid ..	0·833	0·208	0·026	0·0083
Sodium chloride	0·840	0·210	0·42	0·105
Glucose ..	—	2·2	—	1·11

It is interesting to note that monozygous cattle twins show very similar threshold values, thus indicating that taste discrimination is almost entirely genetical in origin and that environmental factors play only a minor role in this type of behavioural reaction. "Taste blindness" in man is recognised from recent investigations as being genetically transmitted and is a feature of the taste receptors.

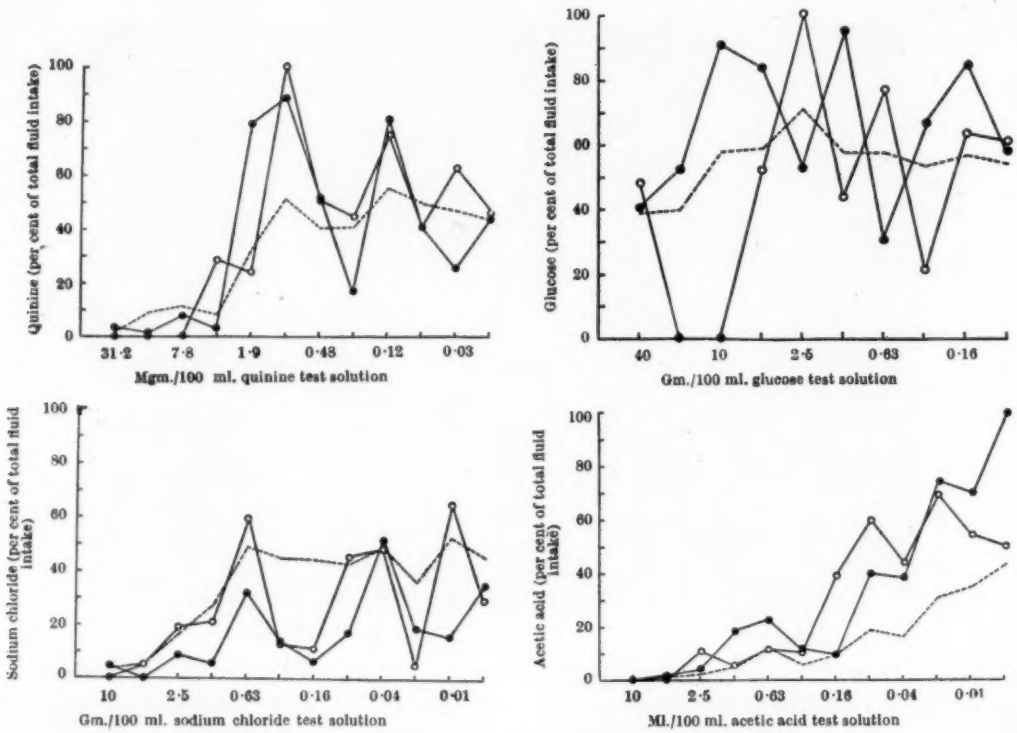


FIG. 4.—Preference tests for monozygous twin calves. The broken line is the mean of all the calves tested. The open and closed circles are the results obtained from the individuals of a monovular twin set, one of which was housed at Potters Bar and the other at Camden Town. (Reprinted with permission from Bell and Williams (1959), *Nature*, **183**, 345.)

It is noticeable in preference tests that very often after attainment of the acceptance threshold the animals continue to prefer diluted solutions of the sapid solutions (see Fig. 3). It is possible that the taste imparted to the weak test solution by its distinctive flavour may increase its palatability, although this partiality did not cause an increase in the absolute amount of fluid consumed during the day.

The slightest contamination of drinking fluid by faeces causes complete and absolute refusal, which suggests that the excreta contains substances which cause complete aversion.

Self-regulatory Functions

Following much careful experimental work, Richter (1942) put forward the concept of total self-regulatory function of the body, whereby an animal reacts to maintain its own internal environment at a constant level by selecting the proper food to suit its physiological state. He showed that when rats were offered the choice between a substance of nutritional value and water, the rats indicated a preference for the substance even when the concentration was very low. Substances such as glucose, sodium chloride, potassium chloride and calcium lactate were preferred in this way in low concentrations; consequently, the selection must have been made by taste. Following adrenalectomy, rats consume sufficient extra salt to maintain good health, and following removal of the parathyroid glands they prefer solutions of calcium lactate. Richter (1935) also showed that removal of the posterior lobe of the pituitary gland with a consequent marked diuresis was not fatal when the rats were allowed access to unlimited supplies of water. Under these circumstances the animals consumed daily more water than their body weight, thus compensating for the loss of the antidiuretic principle. When the gustatory nerves are cut, thus eliminating most of the taste receptors, adrenalectomised rats no longer increase their salt intake and, as a consequence, die (Richter, 1939). This type of experiment shows that taste plays a very important part in the life of the organism by aiding the regulation of the biochemical levels appropriate for health. Richter postulates that the body possesses "total organism regulators" which control the whole physiology of the animal, in the same way that hormones affect certain processes, and that taste plays a major part in this response.

In 1933 Harris, Clay, Hargreaves and Ward showed

that thiamine-deficient rats selected thiamine-containing food when offered the choice of two diets, one with thiamine and one without. Richter and Barelare (1938) have taken this type of experiment further and studied the nutritional needs of the rat during pregnancy and lactation. The rats were given access to eleven substances and when allowed a free choice showed quite definitive and consistent changes in appetite during pregnancy and especially during lactation. Tribe (1955) conducted a similar experiment in rats and failed to confirm Richter's findings. Gordon and Tribe (1951) also failed to show any relationship between the selection of food and the needs of pregnant ewes, for of the experimental animals only one lambed normally. Apparently the poor performance of the ewes was due to the selection of a diet deficient in protein, although it must be mentioned that the ewes were kept indoors and some of them had foot rot.

Richter (1942) performed another series of experiments in which he studied dextrose appetite of rats under the influence of insulin injections. During the period of insulin treatment, the dextrose intake was more than doubled. Gordon and Tribe (1951) using two sheep were unable to confirm this result, but their preliminary experiment may not have been continued long enough. Housed goats when offered the choice of a 1 per cent. solution of sodium bicarbonate and another of tap water show a marked preference for the alkaline solution (Bell, 1959). When these animals are given sodium bicarbonate by stomach tube or directly into the rumen via a fistula, the preference for the sodium bicarbonate is depressed until only very small amounts are taken. During the whole of this period the potassium and sodium blood levels show no deviation from normal limits. Small amounts of sodium carbonate do not produce this reversal of preference; 50 g. per day is the optimal amount. It is interesting to note that in sodium-depleted sheep following exteriorisation of the parotid salivary duct, salivary levels of Na and K can be restored and maintained by a daily dose of 50 g. of sodium bicarbonate (Denton, 1957). Apparently, sodium deficiency in ruminants has a special effect on sodium taste.

Examples of self-regulatory processes are numerous in certain deficiency states of cattle. Thus Theiler, Green and Du Toit (1924) showed that phosphorus-deficient cattle have a preference for bones (osteophagia) and that this is a behavioural reaction to restore the biochemical needs of the body. Feeding bone meal prevented the osteophagia in most cases. Gordon, Tribe and Graham (1954) demonstrated that phosphorus-deficient cattle and sheep showed obvious osteophagia or allotriophagia, but this was not corrected by the selection of a phosphorus mineral supplement when this was offered *ad lib*.

Discussion

The actual process whereby taste receptors are excited remains obscure, although it is likely that some chemical reaction occurs, probably at the surface of the cell or on the hairlike processes of the taste cell. This adsorptive process probably occurs rapidly, for taste receptors can respond almost instantaneously and the magnitude of the response is directly related to the strength of the stimulus. Receptors situated in the circumvallate papillae would not be affected until the sapid solution had penetrated the fossa so that impulses would be directed centrally in varying time pattern. Landgren, Liljestrand and Zotterman (1954) have brought forward evidence which suggests that acetyl choline is concerned in the initiation of taste impulses, as with other receptors.

Although the physiological process of activating any single receptor may be similar in those animals that have taste receptors, there are nevertheless very great species differences in the responses of taste fibres, which may account directly for the variation in taste ability that occurs between animals. In addition, it is known that individual receptor cells may respond to more than one taste quality, e.g., acid and bitter (Pfaffmann, 1941). The distribution of the receptors on the tongue varies within species, sweet and salt receptors being associated with the tip of the tongue in man, while in the dog salt receptors also occur at the tip, but sweet receptors have been displaced to the side of the tongue. It is likely, therefore, because of the species variation in response which occurs in the various receptors and the variation in numbers and topographical distribution, that the taste sensation must depend upon the overall pattern of the total activity of the chemo-receptors and that this pattern must vary in different species.

Whether taste is a simple function of the four well-known classes of taste—sweet, bitter, salt and sour—has not been determined. All species which have so far been tested by means of electrophysiological experiments or by preference tests have receptors which react to these four primary taste modalities. Frings (1948) suggests that primary gustatory modalities occur simply as individual points in a very broad taste spectrum encompassing a very large range of electrolytes and non-electrolytes. "Water taste" as a mechanism in all mammals for the discrimination of water separately from its solutes, as postulated by Liljestrand and Zotterman (1954), obviously does not hold, since some species show no reaction to water (Baldwin *et al.*, 1959). Nevertheless, in those species that possess "water" fibres, these receptors may aid in discriminating between flavours. The studies of Cohen *et al.* (1957) on the reception of gustatory impulses at the cerebral cortex, taken together with ablation studies of this area and interruption of the nervous pathways, show clearly that the interpretation of taste signals is a function of the cerebral cortex.

The evidence derived from preference test techniques is more satisfactory than that resulting from electrophysiological experiments, for it is obtained from normal animals in near-normal surroundings. In the main, however, the data obtained from discrimination tests parallels and therefore corroborates the results obtained using electrophysiological techniques. Striking variations between species occur in rejection and acceptance thresholds, but unfortunately few of the domesticated animals have been investigated by this method. The method has not been extended as yet to many of the perplexing problems of taste. For example, a number of cattle die each year from arsenic poisoning as a result of eating potato haulms treated with arsenite. It is not known whether the cattle are attracted by the haulm itself or

by the arsenic, or by some change that occurs in the haulm as a result of the arsenic treatment. It is possible that self-selection studies could be devised for domesticated animals which could shed light on the complex syndromes associated with mineral imbalance and endocrine insufficiency in the way Richter has demonstrated with rats. The "free choice" system of feeding has been extended to pigs (Evvard, 1915) and to cows (Nevins, 1927) with promising results. Russell and Duncan (1956) mention many instances of the beneficial selection of diet by livestock. Using this method, diseases such as pregnancy toxaemia might profitably bear examination, for although Gordon *et al.* (1954) did offer pregnant ewes a free selection of diets, their negative results can be ascribed to the reduced appetite associated with a disinclination to stand up.

The role of taste in the maintenance of biochemical equilibrium is a most complex one, and it will be difficult to investigate in the modern, highly evolved farm livestock. With rats it is fairly easy to conduct experiments under rigidly controlled conditions, but this is much more difficult to attain with the complicated environment of the domesticated animals. The cost of experiments on the larger animals is always a deterrent, but the use of monovular twins or of litter mates can probably allow significant results to be obtained from small numbers.

Animals grazing on pastures deficient in certain minerals are unable to compensate for this, for their prime need is for calories, and the amount of food that can be ingested is governed by the capacity of the digestive tract. This is the dilemma of cattle or sheep grazing copper- or phosphorus-deficient pastures. The urge developed in the animal to take in food for energy requirements overrides any sensory stimulus that may be obtained from taste receptors. The predominant role of the need for nutrient materials may even force animals to ingest poisonous substances such as molybdenum, arsenic, selenium and fluorine. The disruption in the internal environment and the consequent behavioural changes is well exemplified by the aphosphorosis of cattle which occurs seasonally on certain pastures. Cattle showing this deficiency develop the habit of eating bones, the degree of osteophagia following directly the depression of the blood phosphorus levels. As a rule, cattle avoid bones with flesh still attached to them and show a marked preference for bleached and dried bones. The condition can be treated or prevented by the feeding of bone-meal, but cattle offered phosphorus-rich substances do not normally take sufficient to regulate their phosphorus imbalance. This type of perverted appetite is associated with other deficiency diseases, and may be the manifestation of "drives" emanating from "centres" situated in the hypothalamus controlling appetite and metabolism, or from the cerebral cortex with its regions which control masticatory and lip movements. It is possible that the "drives" associated with perverted appetites may become "learned behaviour", so that even when the deficiency state has been overcome the abnormal behaviour continues. The ingestion of small stones by dogs and egg-eating and feather-picking by poultry may be examples of this form of habit formation. It is also possible for animals to develop abnormal tastes by habituation when there is presumably no basic cause: examples would be the craving for ice-cream shown by some cats and dogs and for mow-burnt hay and silage by cows.

Taste, in addition to its nutritional function, probably has a protective function in that it monitors the quality of food intake. The value of this protective function is correlated with other physiological processes. In the rat, taste is extremely well developed, a biologically important fact since the rat cannot vomit; in the dog, vomiting is well developed so that chemo-receptors in the gastric mucosa serve as a second line of bodily defence. Cattle have many enzymes in the alimentary tract which may destroy toxic substances; for example, Harvey (1958) showed that the dinitrophenols are destroyed by the microflora of the rumen. The avoidance of food material which has been contaminated with faeces is effected by taste and smell and could well be protective in limiting the entry of pathogenic organisms by the alimentary route.

It is likely, therefore, that taste plays an important part in the lives of the domesticated animals in both health and disease. Like the other special senses of the domesticated animals, not enough detail is known of the physiology of taste. There are, however, a number of methods whereby our knowledge could be improved, and although they may be costly they would be very well worth while. The results obtained could have far-reaching implications in animal health, nutrition and animal husbandry, as well as in the fields of animal behaviour and neurophysiology.

References

ANDERSSON, B., and JEWELL, P. (1957). *J. Physiol.*, **139,** 191.
——, LANDGREN, S. OLSSON, L., and ZOTTERMANN, Y. (1950). *Acta Physiol. Scand.*, **21,** 105.
AREY, L., TREMAINE, M., and MONZINGO, F. (1935). *Anat. Record*, **64,** 9.
BALDWIN, B. A., BELL, F. R., and KITCHELL, R. L. (1959). *J. Physiol.*, **146,** 14P.
BEIDLER, F. M., FISHMAN, I. Y., and HARDIMAN, C. W. (1955). *Amer. J. Physiol.*, **181,** 235.
BELL, F. R. (1959a). *J. Agric. Sci.*, **52,** 125.
——. (1959b). Unpublished data.
——, and WILLIAMS, H. L. (1959). *Nature*, **183,** 345.
BENJAMIN, R. M., and PFAFFMANN, C. (1955). *J. Neurophysiol.* **18,** 56.
BREMER, F. (1923). *Comp. Rend. Soc. Biol.*, **89,** 432.
COHEN, M. J., LANDGREN, S., STROM, L., and ZOTTERMAN, Y. (1957). *Acta Physiol. Scand.*, 40, Suppl. 135.
DENTON, D. A. (1957). *Quart. J. Exp. Physiol.*, **42,** 72.
EVVARD, J. M. (1915). *Proc. Iowa Acad. Sci.*, **22,** 402.
FRINGS, H. (1946). *J. Expl. Zool.*, **102,** 23.
——. (1948). *J. Comp. Physiol. Psychol.*, **41,** 25.
——. (1951). *Experientia*, **7,** 424.
GORDON, J. G., and TRIBE, D. E. (1950). *Agric. Prog.*, **25,** 94.
——. (1951). *J. Agric. Sci.*, **41,** 187.
——, and GRAHAM, T. C. (1954). *Brit. J. Anim. Behav.*, 2, 72.
HARRIS, L. J., CLAY, C., HARGREAVES, F. J., and WARD, A. (1933). *Proc. Roy. Soc. B.*, **113,** 161.
HARVEY, D. G. (1958). *J. Comp. Path.*, **68,** 54.
LANDGREN, S., LILJESTRAND, G., and ZOTTERMAN, Y. (1954). *Acta Physiol. Scand.*, **30,** 105.
LILJESTRAND, G., and ZOTTERMAN, Y. (1954). *Acta Physiol. Scand.*, **32,** 291.
NEVINS, W. B. (1927). *Univ. Ill. Agric. Exp. Sta. Bull.*, 289.
PATTON, H. D. (1950). *Ann. Rev. Physiol.*, **12,** 469.
——, and AMASSIAN (1952). *J. Neurophysiol.*, **15,** 243.
——, and RUCH, T. C. (1944). *J. Comp. Physiol. Psychol.*, **37,** 35.
PFAFFMANN, C. (1941). *J. Cell Comp. Physiol.*, **17,** 243.
——. (1953). *Science*, **117,** 140.

PFAFFMANN, C. (1955). *J. Neurophysiol.*, **18,** 429.
RICHTER, C. P. (1935). *Amer. J. Physiol.*, **112,** 481.
——. (1939). *Endocrinology*, **24,** 367.
——. (1942). *Amer. J. Physiol.*, **135,** 781.
——. (1943). Harvey Lectures, Ser. 38. 63.
——, and BARELARE, B. (1938). *Endocrinology*, **23,** 15.
——, and CAMBPELL, K. H. (1940). *Amer. J. Physiol.*, **128,** 291.
RUSSELL, C. P., and DUNCAN, D. L. (1956). Minerals in Pasture: Deficiencies and Excesses in Relation to Animal Health. Commonwealth Agricultural Bureaux, Slough.
TRIBE, D. E. (1955). *Brit. J. Nutr.*, **9,** 103.
WATSON, R. (1944). *Bull. Comm. Sci. Indust. Res. Aust.* No. 180.
WEDDELL, C. H. (1936). *J. Comp. Psychol.*, **21,** 233.
ZOTTERMAN, Y. (1935). *Skand Arch. Physiol.*, **72,** 73.
——. (1949). *Acta Physiol. Scand.*, **18,** 181.
——. (1956). *Acta Physiol. Scand.*, **37,** 60.

THE SPEAKER'S INTRODUCTION

The Chairman, Professor Alex. Robertson, in introducing the author of the paper, recalled that on the previous Sunday the President of the Local Association had said that they were endeavouring at the Congress to provide for every taste—and at the present session they were indeed providing for the taste of domesticated animals! Those who might have thought that the story of the sense of taste was only for those who were supposed to dwell in academic ivory towers would have realised, after listening to Dr. Taylor's fascinating philosophical ponderings the previous day, that there was much more to it than that, and that it held out considerable practical possibilities. It was suggested that they might, if they took up certain lines of investigation, find out a great deal about why animals ate certain things and not others. It occurred to him that if research went far enough, with modern techniques of brain-washing they might even persuade the animals to eat things that they did not want to eat but which were known to be good for them!

Dr. F. R. Bell, in presenting his paper, said that he did not wish to add anything of a material nature to the paper as circulated, but he wished to take the opportunity of saying how fortunate he was in having Mr. Dow to open the discussion, because Mr. Dow had an unrivalled knowledge of animal feeding and the preparation of animal foods.

At present there was little authenticated data on the sense of taste in domesticated animals. In fact, there was very little factual information about any of the 5 senses of domesticated animals, let alone of the hypothetical sixth sense of which they had heard something the other day.

The paper was an attempt to collate what information was available on taste and relate it to the physiology of the animal as a whole. Any animal's physiological processes were directed to the maintenance of a steady state within the body. This was epitomised by the well-known dictum of Claud Bernard that life depended on the preservation of the internal environment. The senses, including the sense of taste of course, were the link between the internal environment and the animal's external environment.

Perhaps he might comment on Dr. Taylor's admitted flight of fancy in which he had foreseen the use of the wine taster's art as a means of selecting the appropriate grasses to be fed to cattle. In a way, this had already been done, because there were reports in the literature of animals—cattle and sheep—being allowed to select from a series of grasses on the cafeteria principle. Unfortunately, the nature of these grasses varied tremendously from season to season and from field to field. Their succulence and their mineral content varied, so that unfortunately it was not possible, as it were, to bottle them and ascribe any form of vintage rating.

In this connection, he thought that they should be very careful in their judgments. They must refrain from being subjective and from being too anthropomorphic. On the other hand, he believed that the domesticated animals had a sense of taste perception very similar to that of man, because the affective behaviour of animals when they were confronted with obnoxious flavours closely paralleled that of man.

Dr. Bell then showed a short film which he had obtained through the courtesy of his friends and colleagues Drs. Andersson and Jewell. The film had been made at the Veterinary High School in Stockholm and illustrated the very high quality of research work on the central nervous system of domesticated animals which was being done there.

The first part of the film showed the effect of electrical stimulation of the central pathways of taste. In the second part of the film, the thresholds recorded by the goats were seen to be altered by the introduction of a lesion within the central nervous pathway. The lesion was produced by the passage of an electrical current between two electrodes which brought about a coagulation of the central nervous system and interrupted the central nervous pathway. As a result of this lesion, there was an alteration in the taste thresholds of the goats.

The Opener

Mr. J. K. D. Dow, who opened the discussion, said that it was his job to try to link the experimental work covered by Dr. Bell in the paper with the everyday practice in this country; there was a very wide gulf between experimental thought and the simple practice which was based largely at this stage on art rather than on science.

Many might wonder what was the importance to the practitioner of studies on the sense of taste. They might also wonder what special competence he possessed to open the discussion. On the first point, he thought they should know that the work Dr. Bell was reporting formed only one aspect of Dr. Bell's much wider studies into the biochemistry of brain metabolism. On the second point, like them, he knew of no reason except perhaps that he was closely associated with an industry which was a major consumer of flavouring agents.

Apart from unknown quantities of coriander, vanilla, peppermint, and protein hydrolysates, about 250 tons of aniseed and fenugreek were consumed in this country by livestock annually. These were all added at the "pinch-per-bag" level and they served only as flavouring agents. About 15,000 tons of cane sugar was also used, partly for sweetening and partly for energy value, and slightly less than half-a-million tons of beet molasses was employed partly for sweetening, partly for energy, but mainly for its well-known binding effect in the manufacture of pellets and nuts. If they disregarded sugar and molasses because their cost could be debited to their dietary energy contribution, the 250 tons of aniseed and fenugreek cost the farming industry about £30,000 per annum. Was that worth while and what scientific basis was there for it?

Previous workers employing laboratory small animals had reached a variety of conclusions which were not always helpful. For example, Cohn had attributed taste largely to sapophore groups in molecules. This was a highly scientific way of saying that he attributed taste largely to taste. However, he had pointed out that with increase in molecular weight, as one ascended a homologous series, the taste of the members gradually changed from sweet to bitter. For example, ethylene glycol was sweet, propolyne glycol less sweet, and hexamethylene glycol bitter. Renqvist, working on esters and acids, had concluded that increasing molecular weight was accompanied by decreasing taste, and Barral and Ranc had said that there was no general law by which the taste of any compound could be predicted. Kodama had reviewed the taste of organic substances; Holleman had summarised the knowledge on saccharines; and Finzi and Colanna had critically surveyed the relation between chemical constitutions and taste. If this did not appear sufficiently complex, one could be further confused by an I.T.V. advertisement which suggested that every coffee bean had its own taste buds!

Dr. Bell was to be congratulated on his efforts to show what was applicable to farm animals. Using goats, he had, for example, demonstrated the results of preference tests against the primary modalities, but there was no reference to temperature effects. Weber had shown that degree of taste sensation was greatest when the test substance was at body temperature, and Komura, measuring taste limits with acid, salt, sugar and quinine, had found that sensitivity doubled between 10 deg. and 20 deg., was fairly steady from 20 deg. to 30 deg., and fell from 30 deg. to 40 deg. Temperature was therefore important, and so was the total

daily nutrient intake in young animals fed milk and milk substitutes, particularly when early weaned. He would therefore be glad of guidance on the temperature for milk or milk substitutes in relation to their palatability. Was the generally recommended blood heat the optimum temperature for calves, or except for calorific considerations would they, like the baby pig, take cold milks equally well?

Further, sense of taste was clearly associated with sense of smell. A simple experiment would demonstrate how much of "taste" was really odour, in the human. If one held one's nose while eating, boiled turnips, apples and onions became almost indistinguishable; ham and lamb were practically the same, and, though he had not risked it, port, he was told, tasted like sugar and a good claret like weak vinegar. Much of the discrimination attributed to the palate was obviously performed by olfactory organs. In view of this, he would have liked to hear from Dr. Bell what his thoughts were, at any rate as an experimental approach, to determining the interaction of small and taste in domestic animals. If the feeding stuffs industry was to justifiably load the farming industry with an extra £30,000 per anuum, it was important that the interactions of smell, taste and sight to be considered and catered for as they were in panel testing and product development for humans. At the moment the literature gave no such guidance, and most work on domestic animals had been conducted as simple preference tests.

Studies on the faculties of birds had been reported since 1835, and the general conclusion, although not unanimous, was that the chicken had a fairly well developed sense of taste which was fundamentally different from that of man. The difference was in the relatively small number of taste buds confined to the base of the tongue and floor of the pharynx, and there was some doubt as to whether they were the sole teste receptors, although morphologically they resembled those of mammals, but the possibility of enzymes or special cell types acting as chemoreceptors must be considered.

Although the chicken could differentiate between certain flavours, it was evidently indifferent to flavours. Kare and others had studied the response of chicks to about 48 different flavours and substances administered in the drinking water. The presence of a taste sense had been definitely established by negative response to some flavours; for instance, costus, pathchouly and sandalwood oil had all been rejected. Each of the acceptable flavours had been well received at a particular concentration and the chicks had been sensitive to small alterations in concentration.

There were no direct references to the pig having an ability to taste, but several workers had described preference tests. The pig definitely showed a preference for certain ingredients, but the response was not consistent and the value of sweetening agents in pig rations had not been established. Stickler commented that the odour of a pig feed probably influenced the buyer more than the pig.

Workers at Cornell University reported having studied several hundred flavours varying from artificial fruits such as cherry, strawberry and grape to aniseed, on different animal species and under a variety of conditions. They suggested that additions of aniseed and cinnamon might be detrimental in certain conditions, and that aniseed oil in calf rations significantly depressed palatability. Synthetic sweeteners had not been found as attractive to animals as to humans, and they had concluded that there was no acceptable evidence that any commercially available feed flavour increased appetite or productivity.

Work at Colworth House covering a wide variety of flavouring agents had, so far, failed to indicate any flavour which did not give rise to flavour "fatigue". By fatigue they did not mean the phenomenon of electrically stimulated experimental fatigue induced by Allen and Weinberg, but the fall-off in daily food consumption after a prolonged period, and following initial increases in test food consumption, as compared with a standard diet. This phenomenon was well recognised by most practical animal husbandrymen and was seen in the waning appetite of housed dairy cattle at the end of the winter season during which the basic diets had been monotonous. Stapleton had also noted it among cattle grazing single species of pure grass stands, and this had given rise to his advocacy of "contemporaneous browse". The problem was dealt with by most stockmen preparing animals for show by continually altering the texture, succulence and palatability of the rations to obtain maximum intake. It was largely because of this art, rather than science, that manufacturers were tempted to use spicing agents, and the feedingstuffs industry as well as individual farmers would welcome any experimental work which would lead to the elimination of that flavour fatigue which accompanied the continued use of the same ingredients, even when they were fresh and initially palatable.

Those were the main points that he wanted to make on Dr. Bell's excellent paper, and he wished to congratulate Dr. Bell on his courageous approach to a difficult experimental subject and to assure him that he carried the best wishes of all stockmen in his attempt to rationalise an ancient art.

The General Discussion

Mr. W. M. Brownlie (Thurgarton): "I would like to congratulate Dr. Bell on introducing a difficult subject which I am sure is going to become increasingly important. One of the difficulties in this field is that of designing suitable experimental techniques that will give significant results and I would like to quote one or two examples in illustration.

"When we were doing some work on free choice of minerals by sheep we put up a range of some 13 mineral elements in bowls on a long covered rack in the field. Each element was incorporated in a base of common salt and the latter formed 98-99 per cent. of the mixture. Ewes with lamb at foot were allowed free access to the minerals in the field and the minerals were weighed back every week and the position of the bowls on the rack was then altered.

"After the first weighing we found that the sheep had taken considerably more iron than any other element and we thought that this might be a deliberate choice since the ewes had lambed a short time before. At the next weighing we found that more boron than anything else had been taken, and this led us to watch the sheep to see if there was some other factor affecting uptake. We then found that they usually walked up to the rack somewhere along its length, sampled various bowls along to the other end of the rack and having reached the end stayed there and licked the contents of whichever bowl happened to be at the end. We moved various bowls to the end, always with the same result, until we put in one containing 20 per cent. boneflour and 80 per cent. salt. Even when the bowl was at the end the sheep avoided it and we came to the conclusion that they were interested only in common salt irrespective of the other mineral elements incorporated, and this led them to select against the boneflour which contained only 80 per cent. of salt.

"We estimated that the ewes and lambs took up to 11.5 g. of common salt per day from the rack, and they often walked straight down to the bottom of the field afterwards to drink from a stream. There did not appear to be any good reason for the sheep voluntarily taking such large quantities of salt.

"The second point to illustrate the importance of the experimental technique comes from work done by one of my colleagues, Mr. H. G. Stockdale. He found that when pigs in the same pen were given a choice of saccharine supplemented and unsupplemented food, they took more of the food containing saccharin, but when pigs in different pens were given either supplemented or unsupplemented food, they ate just as much of one as of the other. This, to some extent, supports Mr. Dow's suggestion that the effect of additives may sometimes be rather more on the farmer than on the animal."

Mr. A. Brownlee (Compton): "I wish to consider the question, raised by Dr. Bell, of the animal's taste reactions in relation to its needs. At Compton a free choice mineral-substance cafeteria showed that cattle would select dibasic sodium phosphate, sodium chloride and also copper sulphate powdered and diluted with kaolin 1 in 10. They did not take manganese sulphate, cobalt sulphate or potassium iodide, each similarly diluted with kaolin. There was no clinical evidence that these cattle were deficient in phosphorus, sodium chloride or copper and it is difficult to believe that need was the motive for the licking of these mineral substances unless some not-understood benefit was derived from their taking excess of these substances."

Dr. D. Luke (Stormont): "I too would welcome Dr. Bell's

views on the question of texture. Certainly very fine mashes are not as acceptable as those of a coarser texture but this may not be a matter of taste only.

"I would like to compliment Dr. Bell on his paper. He is one of the very few veterinary workers in the fundamental discipline of physiology. As veterinary knowledge advances we have to look more and more to the basic sciences on which the health of animals depend, and our fundamental knowledge of animal physiology is all too scanty."

The Reply

Dr. F. R. Bell, in reply to the discussion, said that a large number of points had been raised to most of which, unfortunately, he could not give a clearcut answer.

He thought that Mr. Brownlie of Nottingham was quite right in emphasising that in this type of work one must be extremely careful about the design of the experiment. It struck him immediately that in the experiment which Mr. Brownlie described it should have been conducted in a circular box, thus avoiding the two ends of the trough. He agreed wholeheartedly that one must be extremely careful in taking results of this type of work and talking about them.

Mr. Brownlee of Compton had emphasised that some people had found that the cafeteria system worked, in other words, that the animals had made apparently the selection of the materials that they required; whereas in other experiments the animals had not taken the materials which apparently they required.

Throughout the whole of the discussion the question of texture had been referred to. Mr. Dow had first raised it in talking about the interrelationship of the other senses with taste. He believed quite definitely, as others did, that no single sense was used in the assessment of palatability. The sense of smell was co-ordinated with the sense of taste. There was a very close correlation between smell and taste, and equally there was a close correlation between the sense of touch within the mouth—the question of texture. It was well known, for instance, that the dog would not swallow just anything. It was the sense of touch, perhaps, that was most important in the production of salivary reflexes. If a dog was fed on extremely dry, dusty food, the food was held in the mouth for a long time until sufficient saliva was excreted to produce a consistency which allowed the animal to swallow, in other words, for the normal swallowing reflexes to be stimulated. If the food was succulent, then it was swallowed immediately. So obviously there was a very close correlation between texture and taste, as well as between smell and taste, and probably temperature too.

He had no experimental evidence about temperature, but he felt that animals would take any food material at atmospheric temperature, just as well as they would take food which had been raised to the level of the temperature of the body. He could see no reason why animals should be given warmed-up food. On the other hand, if one was feeding an individual animal, and if that animal refused to eat food at environmental temperature, he would have no hesitation in trying it with food at body temperature, because by some queer quirk of the individual animal that might be what it required. He did not think one could be dogmatic on such points, but he thought that one could generalise and say that all these senses were interrelated and correlated in the assessment of palatibility of the food by the animal.

In the usage of the senses of the animal, he believed that the state of the animal as a whole was important. Mr. Brownlee had mentioned sheep that took a large quantity of sodium chloride. Mr. Brownlee had not produced any evidence—and it would be difficult for him to get it—that these animals were not in fact suffering from a deficiency of sodium or of chloride. The whole of the endocrine system was working to see the animal did take in the food what it required to produce the steady state of its internal environment.

He might mention briefly some experiments they had done in which they had altered the internal fluid environment of the animal by exteriorising the parotic duct of cattle so that the saliva of which was equivalent to about a 1 per cent. sodium bicarbonate solution that was lost to the exterior. Immediately there was a change in the taste thresholds of these animals, and they took very much more sodium chloride than when they were conserving the sodium chloride by reabsorption from the gut.

There were thus many factors affecting the taste and the taste thresholds and the usage of taste by animals. It is extremely difficult to elucidate all the problems at this stage, and it would be necessary to perfect many varied experimental methods to make an assessment of them.

The usage of tasteful substances had been mentioned by Mr. Sambrook and Mr. Dow. He thought that they had been used in agriculture and veterinary medicine because they were used in human medicine. In his opinion, there was no real basis as to why they should be used. Mr. Brownlee of Compton had spoken about animals taking copper sulphate. It might be that they should use simple additives to animal diets such as copper sulphate or sodium chloride or sodium-bicarbonate rather than the more bizarre and exotic substances used as tasteful additives today. As yet they really did not know.

Mr. Dow had mentioned the chemical configuration of various tasteful substances. In the paper he had purposely left out any discussion of molecular forms of various tasteful substances. There was a tremendouns amout of literature on this in the human field and on experimental animals. Unfortunately the results were quite contradictory, and one was unable to make any useful contribution therefrom.

Mr. Dow had made a point about smell and its dissociation from taste. This should not be difficult to do experimentally for one could very easily obliterate or remove surgically the olfactory lobes from the brain of any animal. He saw no difficulty in separating taste and smell.

Mr. Dow and other speakers had referred to the question of fatigue in feeding. It was likely that the same mechanisms affected an animal which was sick and did not want food. He did not know the answer. It could be a matter of some mineral deficiency or it could be a matter of a special stimulation of the taste receptors of the individual animal.

Finally, on the question of faecal contamination, it had been found with goats that if there was faecal contamination then they refused the food. Because of this factor one had to be very careful in designing the experiment. Perhaps the fact that animals would regurgitate and remasticate the rumen contents was again correlated with the question of texture. The stuff they regurgitated was in an extremely liquid form, it was, of course at body heat, and it was quite sweet. He did not know whether any of them had had the chance of tasting rumen contents, but they were most unpleasant to the human mouth, mainly because of being lukewarm and not because of any unpleasant taste. He could imagine that in processing of the rumen contents one would lose most of its water and perhaps by bacterial action any sugar that was present.

The Chairman, in closing the session, said that the discussion had highlighted the value of co-operation between the fundamental scientists and those who were more concerned with practical applications. This was a side of physiological work which was often scamped or neglected, and sometimes either treated as a matter of no importance or as a matter so complicated that it was not worth while starting to investigate it.

It had been amply shown in the discussion that a great deal of value could arise from collaboration between those in the field and those in the laboratory in this territory, and they were very grateful to Dr. Bell for the trouble he had taken in providing such an admirable summary of the present position and indicating the possible application of existing knowledge. Mr. Dow had given the discussion an admirable opening, and all of them were grateful to him.

Listeriosis: A Potential Danger to Public Health

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SUMMARY—1. The symptoms, pathology, bacteriology and epizootiology of listeriosis are discussed, and diagnostic methods are described.

2. Reports of listeriosis in Great Britain are reviewed.

3. During 7 years 27 cases of listeriosis were encountered within a small area of South-west England; 17 were of bovine origin.

4. In this series there were 8 milking cows, of which 2 excreted the organism in their milk.

5. Reports of human infection are reviewed briefly.

6. Public health aspects of animal listeriosis are discussed in relation to the excretion and survival of the organism in meat and milk.

Introduction

LISTERIOSIS (listerellosis) is a sporadic or rarely epidemic disease which, in man and the larger animals, is characterised usually by symptoms of encephalitic origin. The disease becomes septicaemic in poultry and in rodents, but septicaemic spread is uncommon in the larger animals.

The disease is caused by the small motile Gram-positive bacillus *Listeria* (*Erysipelothrix*) *monocytogenes*. Pirie (1927) named the organism *Listerella* in honour of Lord Lister, but the designation was rendered invalid by the prior assignment of the generic title *Listerella* to a mycetozoan organism.

The morphological and cultural characteristics of *L. monocytogenes* resemble those of *Erysipelothrix rhusiopathiae* so closely that Topley and Wilson (1936) suggested that both organisms might be classified together in the genus *Erysipelothrix*; in subsequent editions of their work the organism of listeriosis was designated *Erysipelothrix monocytogenes*. Nevertheless, although both organisms cause somewhat similar changes when inoculated into rabbits, *L. monocytogenes* usually evokes reactions of a more severe nature than those caused by *E. rhusiopathiae*; nor are other animal species equally susceptible to the effects of both organisms. Furthermore, the two organisms are antigenically dissimilar and only *L. monocytogenes* is motile: partisan discussion of the classification of the causal organism of listeriosis is beyond the scope of this paper.

Listeriosis was described first by Murray, Webb and Swan (1926), who investigated losses among rabbits kept for experimental purposes at Cambridge. The disease was identified subsequently in a wide variety of animal species in Australia, Canada, Denmark, Finland, France, Holland, India, Italy, Japan, Malaya, New Zealand, Norway, Russia, South Africa, several South American States, Sweden and the United States of America. Thus, different investigators have recorded the susceptibility of some 30 species of wild and domesticated animals, and Gray (1958b) cited records of the isolation of *L. monocytogenes* from 11 species of poultry and wild birds.

Although epizootic forms of listeriosis have been described in Russia and in the United States of America, studies of the disease have been somewhat neglected in the United Kingdom because here the disease was usually observed only as a sporadic infection. Nevertheless, Paterson (1939; 1940a) recorded outbreaks in sheep and in poultry; Harbour (1941) and Wynn Jones (1954) observed isolated cases in cattle; and occasional human cases have been reported (Gibson, 1935; Wright and McGregor, 1939; Mair *et al.*, 1957; Edmunds, Nicholson and Douglas, 1957).

We have recovered *L. monocytogenes* from cattle, sheep, pigs, poultry and also from a chinchilla. All these animals originated from a relatively small part of South-west England, but other reports indicate that infection exists in most parts of the British Isles.

Because of an increasing awareness of the public health implications of listeriosis in domestic animals, we feel that a review of the present knowledge of the disease is desirable.

Symptoms

Incubation Period

Although the period of incubation in mice infected via the buccal mucosa is about 6 days, a longer period probably elapses when infection is acquired naturally by large animals. Goats may not show clinical signs until 17 to 28 days after experimental infection (Asahi *et al.*, 1957); though Gill (1937) produced typical encephalitic reactions 7 days after instilling cultures into the nostrils of sheep, and subsequently recovered the organism from several parts of the carcasses. Similar results followed injection of cultures into the carotid artery, whereas cultures inoculated by the intravenous route evoked only a severe temperature response which occurred within 48 hours after inoculation.

Clinical Signs

1. Ruminants. The disease commences with a prodromal febrile phase associated with dullness and anorexia. Within 2 to 3 days signs of nervous derangement appear. In sheep and non-lactating cattle the prodromal phase is often missed by the owner, so that nervous symptoms often seem to develop without warning.

The first signs may be slight muscular twitchings or regional stiffness, but obvious incoordination appears quickly. Affected animals wander aimlessly and there is often a marked tendency to turn in circles, always in the same direction ("circling disease") until the animal becomes so weak that it is no longer able to remain on its feet. Some infected animals push against inanimate objects, and a few may show signs of mania. Excessive salivation and nasal discharge occur frequently, and torticollis is not uncommon.

Degenerative changes in the central nervous system (C.N.S.) progress to focal necrosis and cause local paralysis in the muscles whose nerve supplies originate in the affected areas. The facial, abducens, trigeminal and oculomotor areas are affected most frequently, to cause unilateral or bilateral paralysis of the lip with drooling of saliva, flaccidity of the lower jaw, drooping of an ear or marked strabismus. Some animals become blind. Knuckling of the fetlock of one or more limbs occurs occasionally.

Paresis spreads progressively so that the animal becomes recumbent and is unable to rise. Affected animals usually lie on one side and, if turned over, will struggle convulsively to regain their former position. Eventually the animal becomes comatose, but periods of coma often alternate with fits of hyperexcitability when compulsive running movements are made, although the animal is lying on its side.

Abortions have been recorded in cattle (at 4 to 8 months of pregnancy) and in the later stages of pregnancy in sheep. The organism has been isolated from foetuses, placentae and their fluids (Paterson, 1940c; Ferguson *et al.*, 1951; Levi *et al.*, 1953; Stockton *et al.*, 1954). Grey *et al.* (1956) induced abortions in sheep and goats by incorporating large doses of virulent culture in the animals' drinking water.

Wramby (1944) isolated *L. monocytogenes* from two quarters of the udder of a cow suffering from an acute mastitis, and further cases of mammary infection are described in the present paper.

A circulatory monocytosis is found occasionally in ruminants, but this phenomenon is more common in rodents. Whilst samples of the cerebrospinal fluid (C.S.F.), withdrawn via the lumbosacral space, may contain large numbers of mononuclear cells (Wynn Jones (*loc. cit.*), this is not always a feature of the disease.

Neonatal septicaemia is quite common in lambs, but septicaemia is rare in adults. A case of fatal septicaemia was encountered in a 2½-year-old heifer (Case No. 52/212) which died within 24 hours of the onset of symptoms of pyrexia and dullness.

2. Pigs. Affected pigs usually show little systemic disturbance other than signs of an encephalitis. Kosonov and Barsukov (1957) recorded that oedema of the eyelid was a frequent symptom.

Paralysis usually appears first in one or more limbs and progresses centripetally to a fatal termination within 2 to 3 weeks. Occasionally the disease becomes septicaemic and death supervenes rapidly, multiple necrotic foci being found in the parenchymatous organs.

3. Poultry. Listeria infections in birds are usually of the septicaemic type. Symptoms are related to the presence of massive necrotic foci in the liver, the myocardium and elsewhere. The C.N.S. is affected occasionally and paralysis or torticollis result.

4. Rodents. In rodents also the disease occurs as a systemic infection associated with progressive emaciation and oedematous effusions into the body cavities and the subcutaneous tissues.

Stained smears of the peripheral blood usually show large numbers of mononuclear cells, mainly monocytes. In rabbits, leukocyte counts may demonstrate an increase in monocytes from the normal of about 800 to 3,000 or more per cu. mm. during the course of the disease.

Morbidity, Course and Mortality

Sheep appear to be somewhat more susceptible to listeriosis than other domestic animals. Epizootics occur occasionally, but in Great Britain the disease usually only appears in one or two animals in each flock (except in outbreaks of neonatal septicaemia).

Jensen and Mackey (1949) recorded a variation in the incidence of the disease among infected herds of cattle from less than 1 per cent. to 7·5 per cent., with an average incidence of 1·3 per cent.

In both sheep and cattle, if the disease follows a septicaemic course death may occur within 24 hours of the onset of symptoms; more frequently, however, symptoms are progressive and the course is related to the severity of the encephalitic lesions, and affected animals may die only after an illness lasting several days.

The disease usually proves fatal for sheep and pigs, but cattle may recover, though permanent torticollis or other signs of nervous damage often remain.

Pathology

A. Animals

In man and the large domestic animals the predominantly nervous symptoms are referable directly to localised damage in the tissues of the C.N.S., and functional derangement may occur even though lesion are inapparent during macroscopic examination. Oedematous effusions may be found in some cases, and these are usually associated with degenerative changes in the liver and other parenchymatous organs.

Preliminary examination of the contents of the cranial cavity often reveals a mild meningitis, and this is frequently confined to the parts of the meninges which cover the cerebellum, the medulla and the anterior spinal cord. Excess C.S.F. is often present. Although macroscopic lesions cannot be found in all cases, serial sections of the cerebellum, medulla and anterior cord frequently reveal pin-point haemorrhages or even small necrotic areas within the brain substance.

In addition to the low-grade meningitis, histological sections of these parts of the C.N.S. usually demonstrate perivascular oedema, with well-marked "cuffing" by mononuclear cell infiltrations within the spaces surrounding the small blood vessels of the brain and cord (Fig. 1). Although these changes are found principally in the sites mentioned above, they may also occur elsewhere in the C.N.S.

Further evidence of an inflammatory reaction is afforded by focal proliferation of microglial cells and by small areas of degeneration in the brain substance. Some of the degenerate areas show neutrophilic infiltration. Larger zones of complete necrosis may be seen and these are wholly or partly infiltrated by microglia, lymphocytes, monocytes, polymorph neutrophils and, occasionally, by Gitter cells (Fig. 2). If histological sections are stained by Gram's method, *L. monocytogenes* may be visible within the degenerate or necrotic areas, but the organisms may be scanty

and difficult to distinguish even when the disease is well advanced.

In young lambs the lesions are usually those of a neonatal septicaemia with necrotic foci in the liver.

When the disease assumes a septicaemic form, the usual non-specific changes, such as petechial haemorrhages on serous surfaces and excess fluid within the body cavities, may be found together with marked congestion of the parenchymatous organs. Lymph nodes are usually swollen, haemorrhagic and oedematous. When infection is well established, macroscopic examination may reveal multiple small zones of hepatic necrosis. Histological sections show that these necrotic foci tend to be sublobular in extent and are infiltrated by lymphocytes, monocytes and a few neutrophils. Fig. 3 shows lesions of this type in the liver of a young cow (Case No. 52/212), and it is of interest because of the infrequency with which the septicaemic form of the disease appears in cattle.

This animal, to which reference was made previously, was a newly-calved heifer which showed symptoms of acute pyrexia (temperature 107·2°F.) with attendant rapid pulse and respiration. When first seen the animal was salivating slightly and grinding her teeth. A change was noted in the consistency of milk from 3 of the 4 quarters of the udder.

Death occurred in slightly more than 12 hours after the first clinical signs appeared. Autopsy showed a serofibrinous exudate in the abdominal cavity, intense abomasitis, patchy enteritis, multiple focal necroses of the liver, and petechiae and ecchymoses on the serous surfaces of the spleen, intestines and epicardium.

L. monocytogenes was isolated, together with *Escherchia coli*, from the liver and spleen, but not from the bone marrow or from the defibrinated peritoneal fluid. These were the only samples dispatched for laboratory examination. Histological sections revealed inflammatory and necrotic foci of varying sizes throughout the liver substance, resembling closely the lesions produced by experimental infection of rabbits and guinea-pigs.

In spite of the changes in consistency of the milk, *L. monocytogenes* was not recovered in cultures from milk samples collected at the height of the disease.

Lesions in rodents resemble closely those seen in larger animals suffering from the septicaemic form of listeriosis.

B. Birds

Gray (1958b) stated that there are no pathognomonic symptoms or lesions in birds with listeriosis.

A circulatory monocytosis may occur, but this is an inconstant finding in birds which are infected naturally. A fibrinous pericarditis is found frequently and the pericardial sac is distended by fluid. Lesions in the heart consist of massive areas of myocardial degeneration with engorgement of the blood vessels in the peripheral zones; the degenerative changes progress rapidly to necrosis. Occasionally the heart is distorted to produce the appearance of a "round heart" and the degenerative areas may reach 2 mm. in diameter. Necrotic foci are found also in the liver, spleen, lungs and kidneys. The air sacs may show a well-marked inflammation, and ulceration may be found in the ileum and caeca. Conjunctivitis occurs occasionally and a few birds may manifest focal necrosis in the C.N.S.

Pallaske (1941) classified the severity of myocardial lesions on the basis of whether the degenerative changes affected (a) merely a few fibrils, (b) muscular bundles or (c) large areas of myocardium to form massive necrotic zones. *L. monocytogenes* was found at the periphery of the lesions, but not at the centre.

Malewitz *et al.* (1957) infected turkeys by intraperitoneal inoculation and produced in the liver degenerative changes which varied in degree from fatty change to necrosis. The causal organism was found frequently within the Kupfer cells. Perivascular cuffing was evident in the C.N.S. of some of the turkeys.

Diagnosis

In domestic stock, signs of encephalitis, such as continuous walking in circles and flaccid paralysis of the tongue, lip, jaw or ear are all highly suggestive of listeriosis, but accurate diagnosis is often impossible during life. Supporting evidence may be provided by the demonstration of a monocytosis in smears of the peripheral blood; but a monocytic reaction is found more frequently in rodents and occurs only rarely in the large domestic animals. Examination of the C.S.F. may show the presence of large numbers of leucocytes, but this also is an inconstant symptom.

The only satisfactory method of diagnosis is the recovery of *L. monocytogenes* in culture and the demonstration of focal necrosis with perivascular cuffing in the brain or spinal cord. The presence of polymorphs among the infiltrating cells provides further indication of a bacterial aetiology. Confirmation of a diagnosis by isolation of the organism is of particular importance when the disease assumes a non-encephalitic form, though in lambs the presence of multiple necrotic foci in the liver is suggestive of listeriosis and numerous small bacilli are often evident on histological examination of these lesions.

Isolation. L. monocytogenes may be recovered without difficulty from the internal organs of laboratory animals which die of listeriosis after experimental infection. The organism may also be isolated from aborted foetuses of the large domestic animals; but attempts to recover *L. monocytogenes* from the nervous tissue of all species of animals often fail. Possibly a substance inhibitory to the growth of the organism may be present in infected brain tissue; Grey *et al.* (1948) and Osebold *et al.* (1957) recommended that, if initial attempts to culture *Listeria* fail, suspect material should be refrigerated at 4°C. for 45 days before further attempts are made. This technique appears to destroy the inhibitory factor. Whatever the explanation may be, it is always advisable to employ a heavy inoculum of nervous tissue if primary isolation is to be achieved, and tissue suspensions must be ground as finely as possible.

If the presence of post-mortem contaminants is suspected, material may be stored in 50 per cent. glycerol or inoculated into media containing 0·05 per cent. potassium tellurite (Gray *et al.*, 1950); *L. monocytogenes* is able to withstand both substances at these

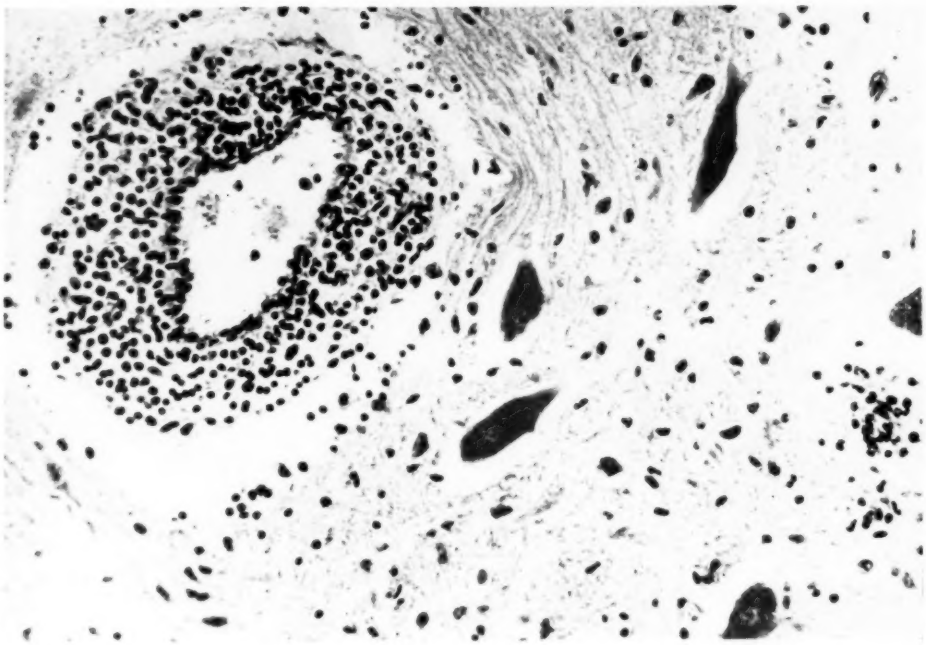

FIG. 1.—Medulla of cow showing marked perivascular oedema and cuffing. H + E × *c.* 250.

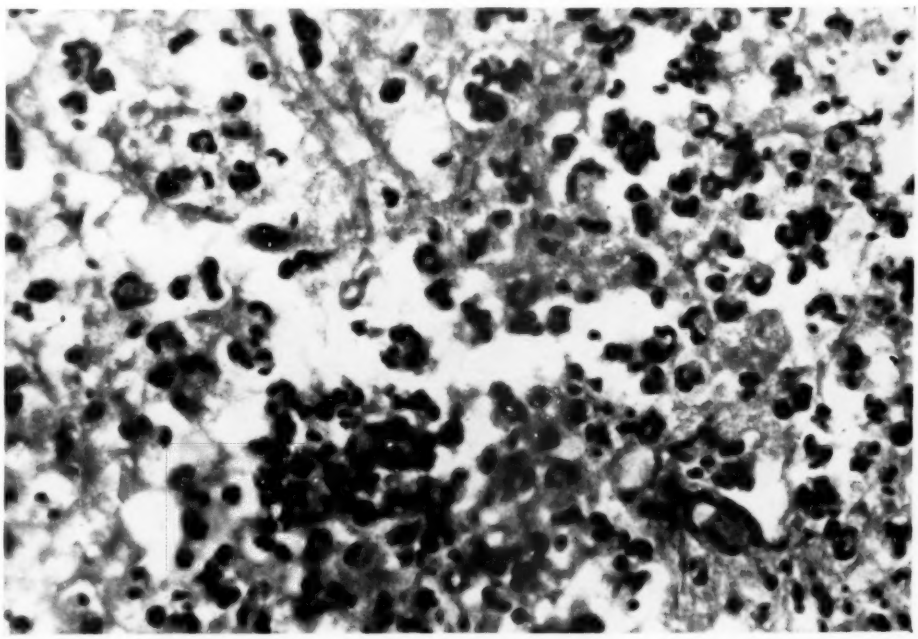

FIG. 2. Thalamus of cow showing neutrophil invasion in area of degeneration. H + E × *c.* 700.

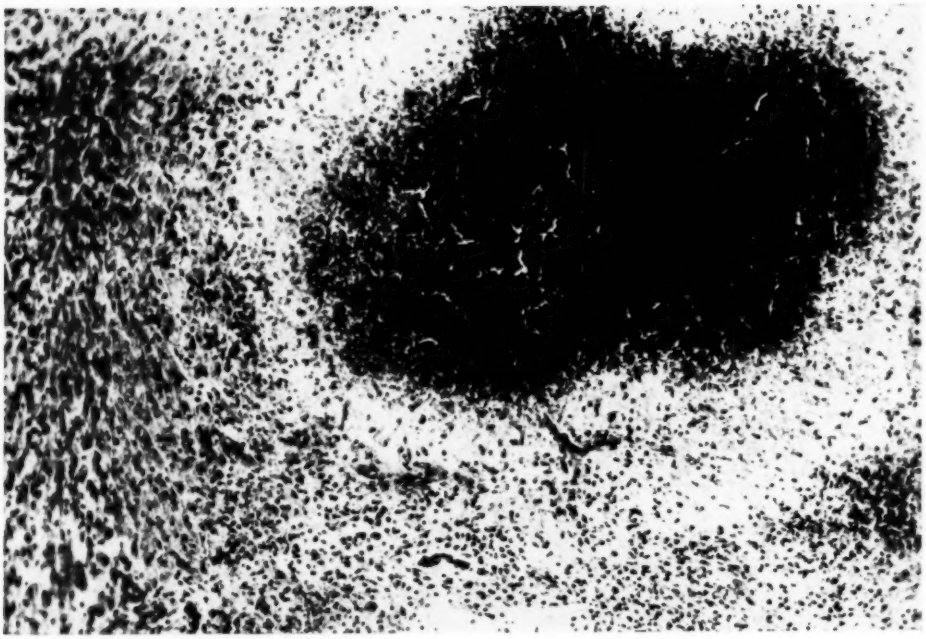

FIG. 3.—Liver of heifer 52/212 showing necrotic lesion. H + E × *c.* 110.

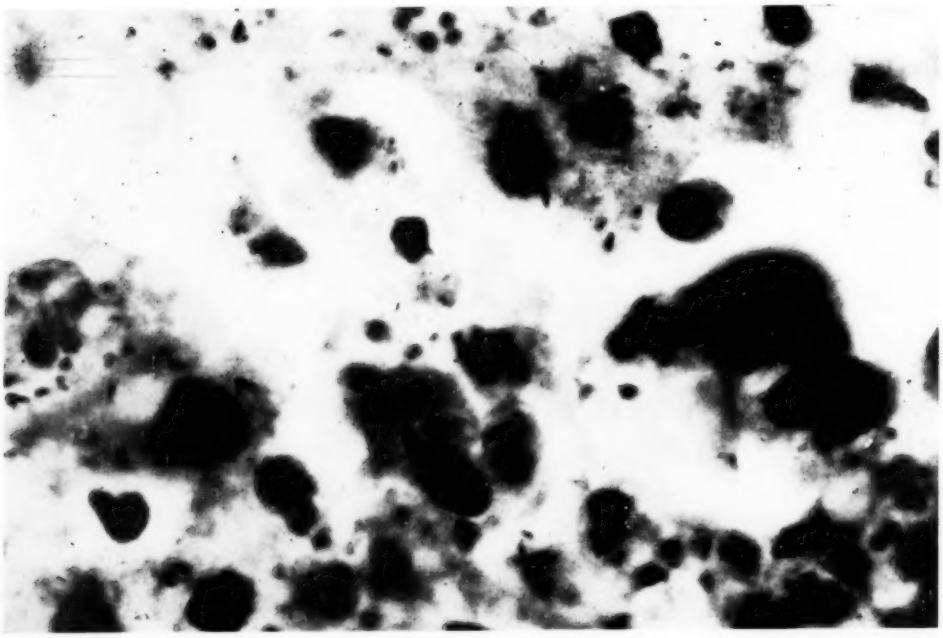

FIG. 4.—Liver of heifer 52/212. Necrotic lesions showing organism. Gram × *c.* 850.

concentrations, whereas most contaminants are rapidly killed or inhibited. Most strains of *Listeria* will also grow on McConkey's medium (Paterson, 1937).

Isolation of *L. monocytogenes* may be facilitated by intracerebral inoculation of fine suspensions of suspect material into rabbits, guinea-pigs or mice. March (1956) advocated the use of mice to obviate the delay caused by refrigeration prior to re-culture.

Instillation of the organism into the conjunctival sac of guinea-pigs or rabbits evokes a specific conjunctivitis and keratitis (Graham *et al.*, 1940; Julianelle, 1941). *E. rhusiopathiae* may cause similar effects, but the reaction is usually milder and keratitis seldom results.

When the disease follows a septicaemic course in ruminants or in pigs, cultures of the small necrotic foci in the liver often reveal *L. monocytogenes* and the organism may be recovered also from the spleen, the kidneys or the lungs.

Morphology. In both direct smears from lesions and in cultures the organism occurs as a Gram-positive bacillus, often curved, which is usually thicker than *E. rhusiopathiae*. Coccobacillary forms are common.

Cultural characteristics. L. monocytogenes grows moderately well under aerobatic conditions, though on occasions primary isolation may be facilitated by at atmosphere containing at least 10 per cent. carbon dioxide. Like *E. rhusiopathiae*, the organism is a facultative anaerobe. Colonies are somewhat larger than those of the related organism and appear white by reflected light.

Both rough and smooth variants exist, but the smooth form occurs most frequently. In the latter form the colonies are convex, transparent and have an entire edge; the organisms occur singly or in short chains. In the rough form colonies have a matt surface with a fimbriate edge, and the organisms are found in long filamentous chains. Paterson (1939) recorded that certain strains develop capsules. Freshly isolated strains usually produce a narrow zone of beta-haemolysis on ox blood agar, but this property may be lost after subculture.

When cultured in glucose broth at room temperature, the organism possesses a peculiar tumbling motility which is imparted by polar flagella. Motility is greatest at about the 4th to 5th hour of growth, but is still marked at 24 hours.

Antigenic Structure. Paterson (1939, 1940a) classified strains of *L. monocytogenes* into 4 sero-types on the basis of their heat labile "H" antigens and secondarily with reference to heat stable "O" antigens. Although agglutinins are detectable in the sera of naturally or artificially infected rabbits, the response of other animals to infection is quite variable, and so serological methods are of little value in the diagnosis of listeriosis. Furthermore, although Patterson's Type 2 was a single strain of human origin and his Type 3 consisted of 10 strains isolated from human cases of infectious mononucleosis in Denmark, there is little evidence that serologically homologous strains manifest specificity for certain host species and are incapable of infecting other potential hosts. Sneath *et al* (1951) observed the same antigens in strains of both human and animal origin, and it would seem that serotyping has little value in epidemiological studies.

Serological tests for the identification of recovered animals have proved unsuccessful (Schwarte and Biester, 1942; Graham, Levine and Morrill, 1943).

Differential Diagnosis

In Great Britain listeriosis is rarely diagnosed in the earlier stages—mainly because of its rarity and the greater incidence of other conditions which pass through a phase of symptoms, varying in length of time, which are difficult to distinguish from those seen in *Listeria* encephalitis. In making a list, we realise that in many instances the typical syndrome of the condition mentioned would not lead to confusion with listeriosis, but that under certain circumstances difficulty could arise in differentiation. In all animals any infective meningitis or meningo-encephalitis or brain abscess will give symptoms similar to those of listeriosis and diagnosis in these cases may well rest on examination *post-mortem*.

Cattle

Hypocalcaemia. Particularly in its more advanced stages the condition produces symptoms similar to those of listeriosis, but there is rarely local paralysis of face muscles. The history of rate of onset and its relationship to time of parturition, together with the result of test therapy, should ensure moderately easy differentiation.

Hypomagnesaemia. In most cases the excitement and hyperaesthesia of hypomagnesaemia is not seen in listeriosis, but in the less acute cases confusion might arise although, once again, details of history and results of test therapy would aid in differentiation.

Acetonaemia. Local paralysis is not a feature of the nervous type of acetonaemia, but, in those cases of listeriosis in which paralysis does not occur, the condition might be diagnosed as acetonaemia. However, detection of ketone bodies in the breath, milk or preferably blood should aid correct diagnosis.

Tubercular Meningitis. The incidence of this condition may confidently be expected to fall below that of listeriosis in the future, but their differentiation must be considered. In general, the meningitis due to infection by *M. tuberculosis* is more chronic and, where an acute stage is seen, excitement may be a feature. A fluctuating febrile reaction is also noted in many cases, whereas this is rarely a feature of *Listeria* encephalitis.

Necrotic Hepatitis. Occasional cases of *F. necrophorus* infection of the liver show nervous symptoms including depression and paresis. Differentiation could be made where jaundice was present during life, but otherwise would rest on post-mortem examination.

Lead Poisoning. The availability of lead in paint, etc., will assist in diagnosis—which may be confirmed by the chemical demonstration of lead in the blood or faeces of the living animal, or in the kidney cortex after death.

Ragwort Poisoning. Poisoning by *Senecio* spp. is often insidious in onset and frequently there is evidence of jaundice and/or diarrhoea. Although affected animals may appear blind and incoordinated,

nervous symptoms are not usually of a paralytic nature. The availability of ragwort and macroscopic and microscopic lesions will confirm the diagnosis in cases of poisoning.

Sheep

Hypocalcaemia. This condition is usually more rapid in onset and is of shorter duration than listeriosis; it is related to lambing in its occurrence and, as in cattle, shows a response to calcium therapy.

Pregnancy Toxaemia. Once again a seasonal incidence may be expected, but the symptoms may be similar to those of listeriosis. Determination of the blood acetone level would differentiate the conditions during life as would the lesions after death.

Coenurus Cerebralis Infestation. In sheep persistent walking in circles is more often caused by this parasite than by listeriosis. The helminth infestation may be distinguished by its slower onset, by its protracted and progressive course, and by the absence of general depression until the later stages of the disease. Softening of the skull over the region of the Coenurus bladder occurs frequently.

Pigs

Middle Ear Disease. This condition is less acute than listeriosis and the head is affected mainly, with disturbance in balance rather than paraplegia. Autopsy reveals specific lesions.

Swine Fever. The epizootic nature and macroscopic lesions are usually sufficient to differentiate this condition, which is, of course, very much more common than listeriosis. In cases of doubt, histological section of the C.N.S., failure to isolate *Listeria* and a positive gel diffusion precipitin test will assist in a confirmation of swine fever.

Aujeszky's Disease. In the pig this disease may be manifested by symptoms attributable to changes in the C.N.S., but these rarely occur in animals over 10 weeks of age, and in older pigs a milder or almost subclinical course is followed. Diagnosis will be made on histological grounds, but presents a little difficulty in some cases.

Teschen Disease. This is usually seen in young piglets. Differential diagnosis may be made on the basis of histological changes and history.

Salt Poisoning. Symptoms similar to those of listeriosis may be elicited, but differentiation may be made on the results of histological examination of the C.N.S., where the typical eosinophilic meningitis is seen.

Tubercular osteomyelitis of the vertebrae may produce paralysis or paraplegia, but rarely more specific symptoms likely to suggest listeriosis.

Streptococcal Meningitis and Arthritis. This condition is commonest in the younger age-groups and normally affects several animals in a group. The presence of arthritis in some of these will assist in differentiation, and the tendency for the febrile phase to accompany the symptoms of meningitis is also a distinguishing feature.

General

Brain tumours are rare in animals. We encountered one case in a cow which gave rise to symptoms similar to, but more prolonged than, those of listeriosis and we record it as a case for differential diagnosis.

In countries other than Great Britain several additional conditions would have to be considered.

Rabies. In view of the danger of human infection and the incurable nature of the disease, any cases in which there is a possibility of confusion should be treated with the greatest caution both *ante* and *post mortem* until histological and biological examination has eliminated the possibility of rabies.

Heartwater. This condition appears to be confined to Africa and its distribution follows that of ticks of the genus *Amblyomma.* Symptoms consist of excitement and incoordination rather than paralysis. In Africa many deaths diagnosed as "heartwater" on clinical grounds are probably due to listeriosis, though the histological changes in the C.N.S. caused by the two diseases are dissimilar and *Rickettsia ruminantium,* the causal organism of heartwater, may often be demonstrated in the endothelium of small blood vessels in the C.N.S. and elsewhere.

Botulism. In its more chronic form botulism may cause symptoms of muscular weakness or a paralysis which is sometimes of a local nature. Nevertheless, in cattle, the paralysis appears to spread from the hindquarters forward and, in fatal cases, it is obviously progressive to a greater extent than in listeriosis. In cattle there is usually a history of depravity of appetite, and the condition occurs in geographically defined areas (Henning, 1949).

Treatment

In many cases, the animal is either presented in an advanced stage of the disease, or not suspected of being infected with *Listeria* until symptoms are so well developed that brain damage must be extensive. In such cases it is not surprising that treatment is rarely successful.

There have been relatively few reports of treatment of large numbers of cases, although Gray and Moor (1953) reviewed the literature and described the results of treatment of a series of naturally occurring cases in cattle and sheep. They used chlortetracycline at a dosage rate of 2·5 gm./day in animals up to 600 lb. in weight, and double this rate for those over 600 lb. They were of the opinion that this was moderately successful, but the results are not as convincing as they could have been because of inability to confirm the diagnosis in recovered animals.

This is a difficulty encountered in all cases of natural infection of an encephalitic type, but the results of treatment of two cases in which the organism was being excreted in the milk, to which reference will be made later, do support the suggestion that the tetracyclines are more useful than the narrow spectrum antibiotics.

We suggest the use of intrathecal injections via the occipitoatlantal space in addition to parenteral inoculation in encephalitic cases.

Epizootiology

Rodents are affected more frequently than any other animal, and among the *Rodentia* the disease is usually acquired by ingestion. Infection is disseminated rapidly by the blood to produce a generalised

infection, and thereafter necrotic foci develop in the liver, the myocardium and in other parenchymatous organs. At one time, *L. monocytogenes* was employed as a rodenticide: but its use was discontinued because epizootics, once started in rats, may spread to other animals and to man. Girard and Murray (1951) suggested that the degree of communicability under these circumstances was only low, but we are unable fully to concur with this suggestion.

Among large domestic animals in Europe the disease occurs sporadically throughout the year, but in the United States of America a tendency for seasonal occurrence has been observed and the incidence appears to be greatest from June to December. There is also a well-marked difference in the conditions under which the disease appears on the two continents. Throughout Europe, and in the United Kingdom in particular, sporadic cases generally occur among sheep and cattle at pasture: whereas in the U.S.A., although outbreaks have been recorded occasionally under open-range conditions of husbandry, the incidence appears to be much greater under "feedlot" management (Olson, 1954) where the morbidity and mortality may be very high. Nevertheless, Osebold *et al.* (*loc. cit.*) recorded an outbreak amongst sheep living principally in the open which reached epizootic proportions in 1951, smouldered enzootically for about 5 years, and then became epizootic once again.

Olson (*loc. cit.*) explained the appearance of sporadic cases as an "opportunist" infection which may lie dormant awaiting the onset of predisposing factors such as cold, stress or intercurrent infection. This hypothesis receives support from records of the occasional recovery of *L. monocytogenes* from tissues of animals dead of other causes (Kerlin and Graham, 1945; Rhodes and Sutherland, 1948; Bolin and Eveleth, 1951). De Lay and Shultz (1954) recorded outbreaks on 11 farms within 4 weeks in one district of California. In explanation of the "spontaneous" outbreaks, it was suggested that some of the sheep on all these premises were symptomless carriers, and that severe outbreaks occurred only when the sheep were herded closely together and exposed to certain predisposing factors. Such factors were considered to be heavy rainfall, dense fog, changes of diet due to supplemental feeding, and the onset of lambing. The outbreak ceased when the sheep were allowed to range over large areas during hot weather with no significant rainfall.

It seems possible, however, that in addition to the disappearance of predisposing factors a gradual increase in the level of naturally acquired resistance may play an important part, if not a major part, in the regression of the disease within infected herds and flocks.

The role of the symptomless "carrier" in the establishment of infection was demonstrated clearly by Russian workers. In one experiment, described by Khalimbekov (1957), 10 clinically recovered animals were placed among 200 sheep and goats known to be free of infection, and the flock was kept in isolation. After 70 days deaths due to listeriosis commenced. Between August and October, 12 animals became affected and, in the following year, a further 18 animals developed the disease throughout the period June to October.

During his investigations, Khalimbekov isolated the organism intermittently from the nasal and ocular discharges, the urine, the milk, and also from the genital organs and semen of infected sheep and goats. Wenkeback (1953) isolated *L. monocytogenes* from human urethral discharges.

It may be concluded that, when infection is introduced into a susceptible group of animals by symptomless carriers, the disease spreads from animal to animal, but clinical signs of infection may only appear when predisposing factors begin to operate. Although the periods of the year differ slightly between the Russian and American reports, a seasonal incidence is discernible in both sub-continents.

Generalisation of infection appears to be infrequent in large animals but not rare, and we believe that dissemination from hepatic foci, via the bile and the faeces, represents the greatest danger to neighbouring animals. Case No. 52/212 is a typical instance in which faecal contamination of the environment probably occurred.

Harbour (*loc. cit.*) recorded an acute fatal infection in a 9-day-old calf. In the light of recent observations, it seems possible that the calf acquired the disease as a milk-borne infection, though no proof of this is available now. The danger of dissemination of listeriosis in milk will be discussed later (*vide infra*).

In addition to direct spread between domestic animals (and man), the disease smoulders among wild animals; and occasionally outbreaks in sylvatic species develop to epizootic proportions, so that outbreaks in wild life are potentially dangerous to domestic stock. The degree of this danger is dependent partly upon the closeness of contact between the particular species of infected wild animal and domestic livestock: but, even when direct contact is normally slight, pasture contamination remains a hazard. This hazard is increased by the tendency for infected wild animals to wander aimlessly, during the period of encephalitis, far from their normal haunts into proximity with man and his domestic stock.

The possibility that listeriosis may spread between wild and domesticated animals was demonstrated by Osebold *et. al.* (1957). These investigators recorded a marked decline in local skunk and racoon populations during an outbreak which appeared first in sheep. During this period several skunks exhibited signs of encephalitis and, at autopsy, two showed lesions indicative of disseminated listeriosis. *L. monocytogenes* was isolated from the bile, liver, spleen, kidneys and urine of one skunk, and the strain proved to be of the same serological type as strains of bovine and ovine origin which were also isolated during this outbreak.

The incidence of listeriosis among the sylvatic populations of Europe remains a subject for further investigations; but it will be remembered that the disease was first described here as a natural infection in rabbits, and Levi (1948) reported that *Listeria* caused deaths from hepatitis amongst voles. Thus, although skunks and racoons have no direct importance here, other animal genera probably play the

same role in the epizootiology of the disease on this side of the Atlantic.

Gill (*loc. cit.*) suggested that the disease was carried by the larvae of *Oestrus ovis* and that *L. monocytogenes* was inoculated into the nasal mucosa of sheep by the anterior hooks of the parasite. The possibility of arthropod-borne infection was generally ignored or discounted during the subsequent 20 years, until Mamedov (1957) showed a correlation between tick infestation and the incidence of listeriosis in cattle. The significance of the observation was confirmed by the isolation of *L. monocytogenes* from the bodies of ticks found on infected cattle. We feel that many other blood-sucking arthropods are *potentially* capable of disseminating the disease mechanically and of inoculating the organism into animals during their feeding period.

The simple exposure of a susceptible animal to *L. monocytogenes* by instillation or feeding often fails to establish clinical infection. This is an important consideration in the epizootiology of the disease, because several ill-defined factors appear either to enhance the pathogenicity of the organism or to facilitate its multiplication once it has entered the animal body. If these factors can be identified and eliminated, listeriosis is unlikely to achieve epizootic proportions even in groups of animals which are susceptible to experimental parenteral inoculation. In addition to the obvious adverse influences such as bad husbandry, cold, damp, physical stress, parturition, etc., many intercurrent infections probably predispose animals to the appearance of clinical listeriosis. Thus, Olson and Seagre (1956) demonstrated a *Listeria*-enhancing factor in the defibrinated blood of infected sheep, and Olson *et al.* (1957) suggested that viruses of the mucosal disease complex may be capable of potentiating not only bovine listeriosis but also ovine infections. Similarly, Gray (*loc. cit.*) incriminated nematodes, salmonellosis, Newcastle disease and the leukosis complex as factors predisposing to outbreaks of listeriosis in poultry. Coccidiosis, coryza, neoplasia and in-breeding have also been suggested as factors which reduce the resistance of birds to latent *Listeria* infections.

The risk of animals acquiring latent infections is increased by the longevity of *L. monocytogenes*; Cole (1941) and Lesbouyries (1943) indicated that the organism may be able to live a saprophytic existence for long periods in contaminated soil. Levi (*loc. cit.*) believed that contaminated soil was a potent source of infection for small rodents.

We have reviewed the disease without special regard to its occurrence in Great Britain, and, to place the matter in true perspective, it must be stated that listeriosis has been recorded only infrequently among animals in this country. Nevertheless, a growing awareness of its presence has resulted in the isolation of *L. monocytogenes* from domestic stock with increasing frequency in recent years.

Within the relatively small area of South-west England from which the laboratories at Langford receive specimens for diagnosis, listeriosis has been identified on 27 occasions since 1952, and many suspicious cases have been seen. Table I records the distribution of the 27 proved cases among animal genera.

TABLE I

CASES OF LISTERIOSIS DIAGNOSED BY THE V.I. LABORATORY AND UNIVERSITY LABORATORY, LANGFORD

Animals Affected	Pre-natal	Post-natal	Total
Cattle	4	13	17
Sheep	0	4	4
Pigs	0	1	1
Poultry	0	4	4
Chinchilla	0	1	1
TOTAL	4	23	27

The majority of cases recorded in Table I were sporadic infections of single animals, but in one of the 4 ovine instances *L. monocytogenes* was isolated from a one-month-old lamb originating from a flock in which a moderately severe epizootic form occurred. During the outbreak 24 of the 280 lambs died and 8 showed obvious clinical signs of infection. An interesting feature of the outbreak was that the disease seemed to be confined to single lambs whose dams received only hay in addition to grazing: clinical infection did not appear amongst twin lambs whose dams received a supplement of kale.

Similarly, one of our 3 bovine cases refers to the infection of several cattle in a herd in North-west Somerset. During the period of observation one cow died of listeriosis, *L. monocytogenes* was recovered from the milk of another, and 5 more showed mild-to-severe signs of nervous derangement. The owner reported that a cow had died after showing nervous symptoms some few weeks previously, but by the time that one of us saw the herd it was no longer possible to establish the cause of death.

It seems unlikely that the incidence of the disease is greater in the south-western part of England than elsewhere in the British Isles; and occasional reports indicate that listeriosis occurs in many areas. Patterson (1937, 1940c) isolated *L. monocytogenes* from poultry in East Anglia and from the livers of ewes in Northamptonshire. Wilson Taylor (1958) reported the occasional isolation of *L. monocytogenes* from animals in South-east Scotland, and Dickie *et al.* (1958) observed two bovine cases in South-west Scotland. In the same area Jarrett *et al.* (1959) recorded an outbreak in piglets. Bracewell (1958) has seen listeriosis in housed sheep in Berkshire. In Yorkshire Watson and Hunter (1958) investigated symptoms of encephalitis in a lamb, and the discovery of multiple necrotic foci in the liver led to a provisional diagnosis of listeriosis. The report of Harbour (*loc. cit.*) indicated that the disease existed in Wales.

It is probable, therefore, that the disease occurs in many or most parts of Great Britain, but that its sporadic nature has resulted in little attention being focused upon the occasional losses that result; and in many instances deaths due to listeriosis have been diagnosed either incorrectly or as "encephalitis of unknown origin".

Public Health

Until recent years, the importance of sporadic infections in man has been small in comparison with

that of the major epidemic diseases. Within the last three decades, control of the latter diseases has thrown into much greater prominence the public health problems arising from "minor" infections such as listeriosis.

Human diseases which are associated with the presence of *L. monocytogenes* have been reported in Great Britain, Russia, Germany, the United States of America and the Scandinavian countries. Listeriosis usually occurs sporadically in human populations, but small epidemics may develop occasionally. Because the mortality rate in untreated cases is about 80 per cent., its infrequent occurrence should not be allowed to mask the seriousness of the disease.

The relationship between human glandular fever (infectious mononucleosis) and listeriosis remains indefinite. Nyfeldt (1929) and Schmidt and Nyfeldt (1938) isolated *L. monocytogenes* from the blood or from the C.S.F. of 4 cases of glandular fever, and lesions suggestive of listeriosis were found in a fifth case. Similar findings were recorded by Pons and Julianelle (1939) and by Webb (1943): but the isolation of *L. monocytogenes* from persons suffering from glandular fever is frequently impossible and doubts have been expressed regarding the role of *Listeria* in this disease. Furthermore, serum from patients in the convalescent stages of glandular fever often fail to show an increase in antibody specific for *L. monocytogenes*; but this in itself is not of great significance because low agglutinin titres have been found in known *Listeria* infections, and similar results have been observed in animals infected experimentally. Evidence is accumulating which indicates that at least one type of glandular fever is caused by a virus, and Van den Bergh *et al.* (1939) were able to reproduce the disease in monkeys by inoculating filtrates of haemolysed infected blood.

Girard and Murray (*loc. cit.*) and Wilson and Miles (1955) believed that the glandular fever syndrome may be evoked both by a virus and by *L. monocytogenes*. In addition, we venture to suggest the possibility that *L. monocytogenes* may lie dormant in man, just as it appears to do in animals, and that in many cases clinical disease supervenes only when predisposing factors begin to operate. Thus, the virus type of glandular fever may be merely one of several such factors.

The pathogenicity of *L. monocytogenes* for man was illustrated by Kaplan (1945), who collected records of 23 proved cases of listeriosis with a further 13 probable cases. Seeliger (1955) tabulated 291 bacteriologically proven cases.

A mononuclear leukocytosis is usually demonstrable in those human infections from which *L. monocytogenes* is isolated and the C.S.F. frequently contains numerous leucocytes of the lymphocyte and monocyte series. In the majority of such cases, the clinical signs are principally of an encephalitic nature originating from a suppurative leptomeningitis and focal necroses in the C.N.S. It is interesting that the meningitis in the human is more suppurative than in animals and that organisms are more easily isolated (Kaplan, *loc. cit.*).

Similar necrotic zones may be found in the liver, but hepatic lesions are found most frequently when prenatal infection occurs (*granulomatosis infantiseptica*): the majority of infected infants are either stillborn or die within a few hours of birth. Thus, lesions are very similar to those found in lambs as described by Jepsen (1942), Gray *et al.* (1949) and Sandvik *et al.* (1958).

Girard and Gavin (1957) observed an apparent increase in human cases and described 4 (3 fatal) instances of neonatal infection. It was suggested that pregnancy predisposes to transient listeriosis in both human beings and in animals, though the mothers of infected infants seldom show well-marked symptoms.

The source and route of human listeriosis has not been determined, but probably both direct and indirect spread occurs. Human beings may contract the disease without any proven contact with animals, though infection has been recorded in animal and human populations on the same premises. Thus, Kornilova (1956) isolated *L. monocytogenes* from numerous domestic animals and birds, and also observed that farm workers may become infected not only directly from sick animals but also as a result of handling aborted foetuses, or even after removing the foetal membranes of apparently normal animals.

Scandinavian reports indicate that *L. monocytogenes* may have been the agent responsible for occasional abortions among the wives of workers in infected farms. Seeliger (*loc. cit.*) recorded neonatal infection in children whose mothers had recently been in contact with sick or dead birds, and poultry may well be a potent source of infection. Paterson (1940b) showed that the organism was able to multiply in embryonated eggs; Gray (1958), in drawing attention to the danger of the dissemination of listeriosis by naturally infected eggs stated that, although eggs laid by infected birds may not necessarily contain the organism, faecal contamination of their shells may constitute a severe risk. Felsenfeld (1951) recorded 2 cases of conjunctivitis among workers in a plant which was engaged in processing birds from an infected flock; *L. monocytogenes* was isolated from the conjunctiva of these workers and also from the spleens of 5 apparently healthy birds.

The observations of Khalimbekov (*loc. cit.*) indicated that, once an animal becomes infected, all its secretions and excretions are potentially dangerous to its fellows and to man. Probably the uncooked meat of animals killed during a period of *Listeria* bacteraemia is almost equally dangerous. Selivanov and Grinitsuina (1958) recovered the organism from meat infected experimentally, and this meat contained viable organisms even after 20 days' pickling in brine. Nevertheless, Slivko (1958) was unable to recover *L. monocytogenes* from the musculature of natural cases, even though it was isolated from the parenchymatous organs and the heart; but it seems likely that none of these cases was slaughtered during a period of bacteraemia.

Although many investigators considered listeriosis to be primarily a disease of rodents and sheep, the majority of our 27 cases occurred in cattle. This is of some considerable importance from the viewpoint of public health workers.

It would appear that "raw" milk from clinical cases, or even from carrier animals, is a particularly

great (though almost unrecognised) danger to human beings, and the ability of *L. monocytogenes* to localise in the udder has not received the attention that it appears to warrant. Instances of mammary infection were recorded by Wramby (*loc. cit.*), who recovered the organism from two quarters of a cow affected with acute mastitis, and by De Vries and Strickwerda (1956), who reported its isolation from the mammary secretions and tissues of a single bovine quarter which manifested a chronic mastitis. The organism isolated in the latter case was of Patterson's Type I.

The danger of milk-borne infection was demonstrated experimentally by Gray and Thorp (1957). Female rabbits were exposed to oral infection either a few days before or immediately after parturition. From 11 does which were infected *post partum*, 7 litters were found to contain one or more infected offspring; and of 16 newborn rabbits whose dams were not infected at the time of parturition, all died within 7 days of suckling infected does.

It seems probable that whenever *Listeria* infections generalise there is a danger of transient infection of the udder; the isolation of *L. monocytogenes* from the milk of 2 of the 17 bovine cases recorded in Table I provides an indication of the frequency with which the organism may be excreted in the milk, particularly as only 8 of these animals were lactating.

In the first instance, at least, infection was far from transient. *L. monocytogenes* was isolated from a single quarter of a cow affected with a fairly severe mastitis. At first the organism was present in association with *Streptococcus uberis*, but penicillin therapy eliminated the Streptococcus. *L. monocytogenes* was isolated intermittently throughout the spring and summer of 1953. Intramammary treatment with chlortetracycline,* given immediately before the cow was "dried off", resulted in the disappearance of the organism from the milk. Milk samples remained free from *Listeria* in December 1953, though *Str. uberis* had returned, and a haemolytic *Staphylococcus* was apparent also.

During the 1958 outbreak in North-west Somerset to which we have referred previously, samples of milk were collected from all animals showing the slightest departure from normality. *L. monocytogenes* was isolated, in pure culture, from the milk of a cow which manifested a mild torticollis and some swelling of the eyelids with a slight convergent strabismus. The animal's gait appeared somewhat incoordinated, but this may have been caused by the torticollis. After parenteral treatment with oxytetracycline,† the organism was not demonstrable in subsequent milk samples.

Although milk from clinically severe cases of mastitis is unlikely to reach the public, we are unable to feel certain that infected milk from mild or sub-acute *Listeria* mastitis is never included inadvertently with normal milk. Because *L. monocytogenes* is able to multiply at ambient temperatures, the inclusion of infected milk would result in the contamination of disproportionately large volumes of normal milk during bulk handling. Furthermore, the possibility of milk-borne infection is increased greatly by those animals which excrete the organism passively in their milk but fail to develop a mastitis. Indeed, the case of the cow from whose milk the organism was isolated during the 1958 outbreak illustrated how clinical signs may be so slight that, but for a prior death or severe encephalitis in another animal, a cow may secrete *L. monocytogenes* and remain undetected.

Unfortunately, *L. monocytogenes* possesses a heat tolerance greater than many other non-sporulating pathogens; and Barber (1939) showed that the organism was capable of resisting moist heat at 55°C. for 30 minutes but not for 60 minutes. This suggested that pasteurised milk should be safe, but Bearns and Girard (1958) showed that pasteurisation is unreliable if the viable organism count before pasteurisation exceeds 5×10^4 *Listeria* per ml. These workers showed that, if the milk contained more than this critical number of organisms, pasteurisation at 61·7°C. for 35 minutes reduced the viable count, but some survivors remained. When the milk was subsequently stored at room temperature, these surviving organisms were able to multiply and, after 48 hours' storage, very high counts (*c.* 10^8 per ml.) were recorded.

In conclusion, *whilst we do not wish to over-emphasise the risk to human beings*, it is essential to recognise that listeriosis in animals may constitute a danger to public health. The accumulated evidence demonstrates that man may become infected as a result of the dissemination of *L. monocytogenes* by vermin, or by direct contact with infected animals or poultry, and there can be no doubt that the infection of milk supplies constitutes at least a potential source of infection for urban populations. We trust that we have been able to show how, by an awareness of this danger, the veterinary surgeon in general practice is able to do much to protect not only agricultural communities but also the general public from exposure to listeriosis.

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* Aureomycin: Lederle.
† Terramycin: Pfizer.

References

ASAHI, O., TETSUYA, H., and YUTAKA, A. (1957). *Amer. J. vet. Res.*, **18**, 147.
BARBER, MARY (1939). *J. Path. Bact.*, **48**, 11.
BEARNS, R. E., and GIRARD, K. F. (1958). *Canad. J. Microbiol.*, **4**, 55.
VAN DEN BERGH, L., LIESSENS, P., and KOVACS, L. (1939). *C.R. Soc. Biol. Paris*, **131**, 156.
BOLIN, F. M., and EVELETH, D. F. (1951). *J.A.V.M.A.*, **118**, 7.
BRACEWELL, C. D. (1958). Personal communication.
COLE, R. K. (1941). *Poult. Sci.*, **20**, 28.
DE LAY, P. D., and SHULTZ, G. (1954). *Proc. Amer. Vet. Med. Assoc.*, 1954, 413.
DE VRIES, J., and STRICKWERDA, R. (1956). *Zbl. Bakt.*, **167**, 229.
DICKIE, A. T., LAUDER, I. M., TAYLOR, W. C., and THORPE, E. (1958). *Vet. Rec.*, **70**, 733.
EDMUNDS, P. N., NICHOLSON, D. N., and DOUGLAS, D. M. (1957). *Brit. Med. J.*, **2**, 188.
FELSENFELD, O. (1951). *Iowa State Coll. Vet.*, **13**, 89.
FERGUSON, L. C., BOHL, E. H., and INGALLS, W. L. (1951). *J.A.V.M.A.*, **118**, 10.

GIBSON, H. J. (1955). *J. Path. Bact.*, **41,** 239.
GILL, D. A. (1937). *Aust. vet. J.*, **13,** 46.
GIRARD, K. F., and GAVIN, W. F. (1957). *J. Path. Bact.*, **74,** 93.
——, and MURRAY, E. G. D. (1951). *Amer. J. med. Sci.*, 221, 343.
GRAHAM, R., HESTER, H. R., and LEVINE, W. D. (1940). *J. inf. Dis.*, **66,** 91.
——, LEVINE, N. D., and MORRILL, C. C. (1943). *Bull. Illinois agric. exp. Sta.*, **499,** 99.
GRAY, M. L. (1958a). *Zbl. Vet. Med.* (Suppl. 1), 110.
——, (1958b). *Avian Diseases*, **2,** 3.
——, and MOOR, G. R. (1953). *N. Amer. Vet.*, **34,** 99.
——, NELSON, R. N., and THORP, F. (1949). *J.A.V.M.A.*, **115,** 103.
——, SINGH, S., and THORP, F. (1956). *Amer. J. vet. Res.*, **17,** 510.
——, STAFSETH, H. J., THORP, F., SHOLL, L. B., and RILEY, W. F. (1948). *J. Bact.*, **55,** 471.
——, STAFSETH, H. J., and THORP, F. (1950). *J. Bact.*, **59,** 443.
——, and THORP, F. (1957). *Zbl. Vet. Med.*, **4,** 405.
HARBOUR, H. E. (1941). *Vet. J.*, **97,** 401.
HENNING, (1949). *Diseases of Animals in South Africa.* 2nd edn. Pub.: Central News Agency Ltd., South Africa.
JARRETT, W. F. H., MCINTYRE, W. I. M., and THORPE, E. (1959). *Vet. Rec.*, **71,** 225.
JENSEN, R., and MACKEY, D. R. (1949). *J.A.V.M.A.*, **114,** 420.
JEPSEN, A. (1942). *Acta. path. et microbiol. Scand.*, **19,** 423.
JONES, E., WYNN (1954). *Vet. Rec.*, **66,** 296.
JULIANELLE, L. A. (1941) *J. Bact.*, **42,** 367, 385.
KAPLAN, M. M. (1945). *New Engl. J. Med.*, **232,** 755.
KERLIN, O. L., and GRAHAM, R. (1945). *Proc. Soc. exp. Biol. and Med.*, **58,** 381.
KHALIMBEKOV, M. M. (1957). *Veterinariya, Moscow*, **34,** 17 (cited *Vet. Bull.*, **28,** 114).
KORNILOVA, A. L. (1956). *J. Microbiol., Moscow*, **27,** 68.
KOZONOV, N. A., and BARSUKOV, G. F. (1957). *Veterinariya, Moscow*, **34,** 23 (cited *Vet. Bull.*, **28,** 286).
LESBOUYRIES, G. (1943). *Rec. Med. Vet.*, **119,** 145.
LEVI, M. L. (1948). *Vet. J.*, **104,** 310.
——, SHAMIR, A., NEEMAN, G., and NOBEL, T. (1953). *J.A.V.M.A.*, **122,** 490.
MAIR, HELÈNE J., MAIR, N. S., STIRK, E. M., and REID, A. W. (1957). *J. Clin. Path.*, **10,** 231.
MALEVITZ, T. D., GRAY, M. L., and SMITH, G. M. (1957). *Poult. Sci.*, **36,** 416.
MAMEDOV, A. A. (1957). *Veterinariya, Moscow*, **34,** 38 (cited *Vet. Bull.*, **28,** 285).
MARCH, R. W. (1956). *Cornell Vet.*, **46,** 274.
MURRAY, E., WEBB, R. A., and SWAN, M. B. R. (1926). *J. Path. Bact.*, **291,** 407.
NYFELDT, A. (1929). *C.R. Soc. Biol., Paris*, **101,** 590.
OLSON, C. (1954). *Proc. Am. vet. Med. Assn.*, 1954, 450.
——, GRACE, O. D., SEAGRE, D., and BLORE, I. C. (1957). *Amer. J. vet. Res.*, **18,** 303.
——, and SEAGRE, D. (1956). *Amer. J. vet. Res.*, **17,** 235.
PALLASKE, G. (1941). *Tier. Wschr. B. & M.*, **37,** 441.
PONS, C. A., and JULIANELLE, L. A. (1939). *Proc. Soc. exp. Biol.*, **40,** 360.
PATERSON, J. S. (1937). *Vet. Rec.*, **49,** 1533.
——, (1939) *J. Path. Bact.*, **48,** 25.
——, (1940a). *Ibid.*, **51,** 427.
——, (1940b). *Ibid.*, **51,** 437.
——, (1940c). *Ibid.*, **51,** 441.
——, (1940d). *Vet. J.*, **96,** 327.
PIRIE, J. H. H. (1927). *Publication of S. Afr. Inst. med. Res.*, **3,** 163.
RHODES, H. C., and SUTHERLAND, A. K. (1948). *J.A.V.M.A.*, **112,** 451.
SANDVIK, O., MOLMEN, K., and KVAAL, M. (1958). *Nord. Vet. Med.*, **10,** 17.
SCHMIDT, V., and NYFELDT, A. (1938). *Bull. Hyg.*, **13,** 528.
SCHWARTE, L. H., and BIESTER, H. C. (1942). *Amer. J. vet. Res.*, **3,** 165.
SEELIGER, H. (1955). *Listeriose.* Pub.: Barth, Leipzig.
SELIVANOV, A. V., and GRINITSUINA, G. A. (1958). *Veterinariya, Moscow*, **35,** 37 (cited *Vet. Bull.*, **29,** 161).
SLIVKO, V. V. (1958). *Veterinariya, Moscow*, **35,** 39 (cited *Vet. Bull.*, **29,** 168).
SNEATH, P. H. A., ABBOTT, J. D., and CUNLIFFE, A. C. (1951). *Brit. med. J.*, ii, 1063.
STOCKTON, J. J., NICE, L., CARPENTER, W. S., and GRAY, M. L. (1954). *J.A.V.M.A.*, **124,** 102.
TAYLOR, A. WILSON (1958). Personal communication.
TOPLEY, W. W. C., and WILSON, G. S. (1936). *The Principles of Bacteriology and Immunity*. 2nd edn. Pub.: Arnold, London.
WATSON, W. A., and HUNTER, D. (1958). *Vet. Rec.*, **70,** 1189.
WEBB, R. A. (1943). *Lancet*, **ii,** 5.
WENKEBACK, G. K. *Proc. 6th Internat. Cong. Microbiol, Rome*, Vol. 2, 669.
WILSON, G. S., and MILES, A. A. (1955). *Topley and Wilson's Principles of Bacteriology and Immunity*. 4th edn. Pub.: Arnold, London.
WRAMBY, G. O. (1944). Cited *Vet. Bull.*, **18,** 294.
WRIGHT, HELEN A., and MCGREGOR, AGNES R. (1939). *J. Path. Bact.*, **48,** 470.

THE SPEAKERS' INTRODUCTION

The Chairman, Professor C. S. Grunsell, said that a feature of Congress for a number of years had been the inclusion of a paper on a disease transmissible to man. These papers had always been well received and had proved most useful in keeping the profession alert to its important responsibility in this respect. Today they were to hear about a disease caused by *Listeria monocytogenes*. It may have been noted that the title used in the provisional programme was "Listeriosis: a menace to public health." There was a difference between "a potential danger" and "a menace". Most of them might be described as potential dangers on the road, but few, he hoped, would be described as a menace.

Indeed, he thought that one of the main objects of presenting the paper was to keep listeriosis as a potential danger and to prevent it becoming a menace to public health.

Mr. A. D. Osborne, introducing the paper, said that he and Mr. Hyslop were indeed worried about the original title and felt that it was a gross overstatement of the case. Even with the title in its present form they realised that the condition was not one which was likely, at present at any rate, to rank in seriousness with many other diseases which were transmissible by means of milk or meat from animals to man—for instance, salmonellosis and brucellosis—but the fact that it was relatively infrequent might tend to its being overlooked.

The incidence of the condition appeared to be relatively high in the South West, and the sporadic cases or small outbreaks recorded in sheep and cattle had occurred almost wholly in the three counties of Gloucestershire, Somerset and Wiltshire. The fact that the organism had been isolated from milk on 2 occasions, in one of which there was no indication in the consistency of the milk that anything was abnormal in the udder, and the recent report of Bearns and Girard that under certain conditions the organism withstood the effects of pasteurisation, suggested that the disease might have a public health aspect.

Twenty, or even 10 years ago they might not have felt justified in bringing the subject forward for discussion, but the tremendous advances towards eradication or minimisation —eradication was sometimes a dangerous word to use—of the major infectious diseases by the medical profession, had directed attention to the more sporadic conditions. Whenever the veterinary profession could anticipate a risk of infection spreading to human beings, and thus reducing the danger, the action should be taken.

The other reason for bringing the paper forward for consideration was the fact that the condition, mainly because of its rarity of occurrence, was difficult to diagnose in the field. Therefore, it seemed logical to assume that many cases were missed. In fact, practising veterinary surgeons had expressed this view after encountering a case in which diagnosis was confirmed.

Whilst symptoms were by no means diagnostic, the original name given to the disease in sheep by Gill nearly 30 years ago—circling disease—still gave a good guide. This was undoubtedly a common symptom in ruminants. It was one which, particularly in cattle, tended to be strongly suggestive, especially when associated with strabismus or facial paralysis. Paralysis associated with the trigeminal nerve might be so marked that, as occurred in one case, the whole lower jaw may be moved from side to side with no resistance on the part of the animal.

Mr. Osborne showed a series of slides illustrating various aspects of the disease.

Bacteriological diagnosis was generally possible, particularly if refrigeration of the brain, or mouse inoculation, was carried out, but on occasions the organism might not be isolated even though lesions were typical. It was interesting to note that the organisms were rarely numerous in brain sections, though easily identified in the septicaemic form and in aborted foetal stomachs. The best characteristics of the organism from a diagnostic point of view were, in their opinion, morphology, motility at room temperature in glucose broth, and the type of lesion produced in the eye of the guinea pig and the liver of mouse or rabbit.

Mr. N. St. G. Hyslop said that after Mr. Osborne's dissertation it only remained for him to deal very briefly with the epizootiology of listeriosis. Several people had told him that they had always thought of the disease as a condition which was exotic to these islands and which was confined to sheep in New Zealand. Those who had persevered with the paper to the extent of reading to the bottom of page 1 would have seen that the disease was almost cosmopolitan and that it affected many species of animals.

Others had asked why it was that the disease had only recently appeared in this country. This was not strictly true, because it was in this country in 1926 that the disease was first described by Murray, Webb and Swan. Due, however, to the natural preoccupation of the medical and veterinary professions with the "major" infectious diseases, reports of listeriosis during the succeeding 10 years, or even longer, failed to make much impact. However, in the latter part of the 1930s Paterson studied, quite extensively, numerous strains isolated from animals and from poultry; and it was this work of Paterson's which had done much to stimulate veterinary interest in the disease.

To his mind, one of the most interesting features of listeriosis in this country was its apparently widespread distribution despite its generally sporadic manifestation. Cases had been recorded from places as far apart as Berkshire and Wiltshire, Somerset and Scotland, East Anglia and Wales. Thus, wherever members may practice, there was a good possibility that if they looked hard enough, and perhaps for long enough, they would eventually find a clinical case of listeriosis.

In considering the epizootiology of listeriosis, it should be borne in mind that the disease was probably capable of being transmitted freely not only between animals of the same or different species, but also that it might be acquired by man. Russian and other workers had isolated *L. monocytogenes* from urine, faeces and milk and also from the genital secretions of infected animals. Thus, during an outbreak, there might be wide dissemination of the organism. The ability of this organism not only to exist on simple substrates, but also apparently to thrive at ambient temperatures, probably permitted it to live a saprophytic existence, and several workers had indicated that ground may remain contaminated for some years.

Although infection might be acquired *via* small wounds in the skin, it was probable that the majority of outbreaks were initiated by the ingestion of the organism. As one might expect, the most severe outbreaks were seen when animals were crowded together, for example in "feedlot" conditions in the United States.

Alternatively, however, clinical manifestations might be delayed until the necessary predisposing factors began to operate; in other words, the disease was dormant. Several workers had isolated the organism from apparently perfectly normal animals and it seemed probable that in such animals the disease was in a quiescent state awaiting the onset of predisposing factors. These factors were very diverse, and the principal ones were mentioned in the text of the paper.

It was of interest to note that Olson and his co-workers believed that viruses of the Mucosal Disease Complex, which are usually considered to affect cattle only, were capable of potentiating listeriosis not only in cattle but also in sheep. It seemed possible that other viruses which are usually considered to be non-pathogenic for an animal species might in fact be capable of acting in this way.

In addition to the obvious source represented by purchased carrier animals, it was possible that infection could be brought on to premises by workers and by contaminated feedingstuffs and so on. Probably one of the greatest dangers was dissemination by rodent vermin and possibly also by other small sylvatic animals such as rabbits, voles, hedgehogs and moles. In addition to transmission by these animals, it seemed possible that insect vectors might play some part.

He did not propose to deal with human infections, as Dr. Mair was to open the discussion and he was much better qualified than the speaker to deal with that aspect. It would suffice, therefore, if he said that although many human cases had had no obvious direct contact with animals, observations in the United States, Russia and Germany, clearly showed that human beings might become infected as a result of handling sick animals or birds, and that infection might be acquired indirectly by man coming into contact with the faeces or discharges of his domestic livestock. Aborted animal foetuses were occasionally infected with *L. monocytogenes*, and even normal placentae might occasionally be dangerous to handle. He stressed the word "occasionally", because the incidence of abortions in animals due to listeriosis was not known, but they do occur.

Finally, it was necessary to consider the dangers associated with animal products. Some Russian workers had shown that after meat had been deliberately contaminated with *L. monocytogenes* it remained infected for quite long periods. On the other hand, other workers had been unable to isolate the organism from the meat of known cases of listeriosis. All that could be said in our present state of knowledge was that the meat of animals which were slaughtered or which died at the time when they were undergoing the bacteraemic phase was probably dangerous. In those countries where the standard of meat inspection was even lower than it was in many parts of this country, meat might represent a possible source of danger: it was to be hoped that it could not happen here.

They felt that another risk to people who were not normally in contact with animals might be that of milk-borne infection. There was probably not very much danger of milk from acute cases of mastitis of any type being used for human consumption. However, they could not feel happy about the chronic and particularly the sub-clinical Listeria infestations of the types cited. They felt that milk might be just one of several sources of human listeriosis in urban populations.

The danger was exerbated on the occasions when infection of milk did occur—and these were probably infrequent—by the fact that the organism was able to live and multiply at room temperature, so that a single cow might contaminate quite a large quantity of bulked milk. In addition to this, of course, the organism was able to withstand pasteurisation temperatures of at least about 60 degrees for half an hour. There was a considerable drop in the viable count but, provided that the original contamination was sufficiently great, some would remain and then, when the milk cooled to ambient temperature, the survivors were able to multiply again. This, again, was a source of potential danger.

He hoped that they had been able to suggest some of the dangers of this disease to both animals and man. The problems that faced the veterinary profession were identification of the symptomless carrier, the rapid diagnosis of listeriosis in those animals which showed only mild and indefinite symptoms and, of course, the complete sterilisation of milk from those farms on which it was known that the disease existed.

The Opener

Dr. N. S. Mair, opening the discussion, said that when he was first asked to open the discussion he got the impression from Mr. Hyslop that he would have to make only a few remarks. He thought that was a very good idea, as he was just about to go off on holiday. He had hoped to emulate to some extent the sheik who crept into the lady's tent at dead of night and said, "I did not come here to talk"; but, as correspondence between Pirbright and Leicester grew, he discovered that, instead of making a few opening remarks, he had a paper to deliver. He blamed Mr. Hyslop for that. His persuasion was gentle and very insidious. Nevertheless, he was very glad to have the opportunity to join Mr. Hyslop and Mr. Osborne in talking about this condition because there had been a tendency to neglect the work in this country, and most of it seemed to have been done by American and Continental workers, chiefly the Germans and Russians.

Although *L. monocytogenes* was first isolated 30 years ago, to the medical bacteriologist this organism had always been something of a bacteriological curiosity. It was only in the last decade that he had become more aware of its importance as a cause of disease in man. On the other hand, the veterinary bacteriologist had long recognised its importance as a cause of sporadic infection and epizootics in domestic and wild animals. To date, listeriosis had been shown to affect at least 32 species of animal and had been reported from 28 countries.

Listeriosis in man was first reported in 1929 by the Danish bacteriologist Nyfeldt, who isolated the organism from the blood of a 17-year-old Danish boy suffering from infectious mononucleosis—a disease characterised by septic angina and a blood picture in which mononuclears were present in large numbers. In the same year he recovered the organism from 2 more patients with the same syndrome, and a year later he was able to confirm his findings following a small outbreak of the disease affecting 8 more patients who appeared to have drunk infected milk. Since then different forms of listeriosis had been classified broadly into 6 groups.

Dr. Mair then showed a slide which illustrated the types of human listeriosis. Those who were mathematically inclined would notice that the percentages totalled 101. The percentage in group 5 should read 32 and not 33.

The first group was septic-angina with mononucleosis. This was the first recognised form of human listeriosis. All Nyfeldt's patients had a severe angina, often with greyish white exudate, swollen glands, fever and a characteristic leucocytosis with mononucleosis.

Up to about 1957, 90 cases were reported, only one of them from England. That was reported by Webb in 1943, and the patient was a medical student.

A good deal of controversy arose when Nyfeldt announced his findings, since many workers failed to isolate the organism or to find any serological evidence of infection in typical cases of infectious mononucleosis. It was now recognised that there was probably more than one cause of infectious mononucleosis. In this country, the most common cause was a virus infection which, like *L. monocytogenes*, induced a mononucleosis, but, unlike the bacterium, the virus was able to produce a high titre heterophil antibody to sheep red cells. This ability of the serum to agglutinate sheep red cells formed the base of the Paul Bunnell test. About ¼ of the cases of virus infectious mononucleosis had a titre of more than 1/160 with the Paul-Bunnell test. Some cases of listeriosis produced a trace or minor reaction with this test.

In addition to sporadic cases of Listeria-induced infectious mononucleosis, small outbreaks had also been reported. Bilibin, in 1949, observed in the U.S.S.R., 12 cases of acute infection in people who lived in a milieu heavily infested with mice. Urbach and his co-workers had described in 1955 a typical outbreak of infectious mononucleosis in school children, affecting about 26 of them. *L. monocytogenes* was isolated from the throat washings of several patients.

About 11 per cent. of the cases of listeriosis took this form.

The second group was the oculoglandular form. In the septic angina form of listeriosis, there was occasionally an accompanying conjunctivitis, but this was not a predominant symptom. On the other hand, a type of infection did occur in which there was a localised acute, or sub-acute, inflammation of the conjunctiva such as one saw after experimental infection of the conjunctiva in animals. Indeed, the Anton Test, or the conjunctivial test, in which a suspension of the organism was instilled into the conjunctival sac of the rabbit to produce a purulent conjunctivitis, originated from the accidental infection of the eye of a laboratory assistant. He developed a purulent conjunctivitis which responded well to protargol. As far as he was aware, no case of this type had been recorded in this country. A few cases had been recorded in Canada and the U.S.A., but most of the cases had occurred in Russia. One outbreak in that country, reported in 1950, involved 8 children and 1 adult who developed purulent conjunctivitis and definite enlargement of the parotid and submaxillary glands after being in contact with Listeria-infected animals.

The third group was the septic-typhoid type. This was a visceral type of infection and in untreated cases it showed a high mortality. In all about 125 cases had been reported, nearly 100 of these from the U.S.S.R.

Cases included pneumonia with empyema and pyrexia of several weeks' duration. In Norway, a farmer who infected himelf while cleaning out his sheep pen, died of a septic meningitis. An autopsy showed that there was a purulent bronchopneumonia of the left lung. *L. monocytogenes* was isolated from several of his sheep, which were found to be seriously ill. The infection must have been acquired through the inhalation of infected dust. This form of infection could be compared with fever, which could be acquired by the inhalation of dust infected with *Coxiella burneti*, the main host of which was the sheep.

In Holland, in 1952, a case was described of a farmer who became infected while attending the abortion of a cow. Lymphangitis of the right arm occurred, followed by septicaemia and meningitis. Another case involved a Dutch veterinary surgeon who developed a rash and a high temperature. *L. monocytogenes* was isolated from the skin lesions.

Holch, in Germany, had reported the case of a Polish farm labourer who died of *Listeria septicaemia*. The *post-mortem* revealed that there were multiple abscesses in the liver.

The fourth group was the infection of the central nervous system. Until fairly recently infection of the C.N.S. had always been considered to be the most prevalent type of listeriosis, the majority of the cases taking the form of a purulent and often fatal meningitis. Cases of meningoencephalitis and encephalitis had also been reported. The dramatic nature of meningitis and the ease with which it was recognised probably accounted for the conception of some workers that *L. monocytogenes*, like the meningococcus, was particularly neurotropic, but it was more likely that infection of the C.N.S. was the final expression of a generalised septicaemia, similar to that found in T.B. meningitis in children. At *post-mortem* of these cases, bacteria were found not only in the brain, C.S.F., and spinal cord, but also in the liver, spleen, kidney, blood and cardiac muscles. *L. meningitis* could not be distinguished clinically from other bacterial infections, but only by bacteriological diagnosis.

In neonatal cases and in infants, the early symptomatology might be particularly misleading. The baby exhibited shallow and rapid respiration, cyanosis, pyrexia and refused its food. There must be many infant deaths which were diagnosed as due to cardiac or respiratory failure, when a *post-mortem* examination would reveal a Listeria meningitis.

The conception that listeriosis might occur, and be missed, during the neonatal period and in early infancy, was investigated by Lang in Germany. He examined the serological reactions of children with infections of the C.N.S. of known and unknown origin. In 87 children in whom the aetiology was unknown, he found that 43, or 50 per cent., had a titre of over 1/160 to *L. monocytogenes*, and of these 43, 24 had a C.F.T. titre of 1/5 or over. Of 50 children with infection of known aetiology (other than Listeria) only 5, or 10 per cent., in contrast to the 50 per cent. with unknown aetiology, had a titre of 1/160 or over, and of these 5 only 1 had a significant C.F.T. titre.

Later, Lang investigated 241 children with defects of the C.N.S. of unknown origin, and found that 18·3 per cent. showed a titre of 1/320 or higher for Listeria and also a + C.F.T. No such titres were found in 89 cases of known origin. Investigations like these indicated that listeriosis was probably commoner than was realised.

The fifth group was granulomatosis infantiseptica. This condition was probably the most interesting of all the forms of listeriosis, and accounted for about one-third of the cases so far reported. As its name suggested, it occurred in the newborn and in infants, and was characterised by the formation of septic granulomta in the liver, spleen, lung, mucous membranes and sometimes the skin. It was an extremely severe disease and, since it was rarely recognised during life, the diagnosis was often only made at *post-mortem* examination. Even then, it might be mistaken for pseudotuberculosis or miliary T.B. unless a careful bacteriological examination was made.

The conditions was first described by Burns in the U.S.A. in 1934. Thereafter, there was silence for 17 years, and then, in 1951, the disease was rediscovered by Potel and his co-workers in Germany. In 20 babies that had died at birth, or were premature or had a bacterial infection, they found

a pathological picture suggestive of pseudotuberculosis or miliary T.B. Potel was able to isolate an organism from miliary nodules which he called *Corynebacterium infantiseptica*. In the same year, Seeliger identified the organism as *L. monocytogenes*.

The importance of Potel's findings lay in the fact that he was able to recognise granulomatosis infantiseptica as an infectious cause of abortion, still-birth, prematurity and neonatal death. More than 200 cases had been recorded in Germany, 40 cases in Czechoslovakia and a few in Canada. In Czechoslovakia and Canada the mortality had been 100 per cent. The Germans, who seemed to be more on their toes when it came to recognising the conditions, claimed a 65 per cent. mortality. In England, 2 cases had been recognised, though as far as he was aware they had not so far been published. Infection was apparently conveyed in different ways: either *via* the mother's circulation through the placenta, or from the foetus swallowing infected amniotic fluid, or aspirating the fluid during delivery.

A condition similar to that found in infants had sometimes been found in adults. Seeliger came across a case in a 50-year-old shoemaker in Malmedy who, after an illness lasting 6 months, died in a hepatic coma, and the illness was provisionally diagnosed as hepatomegaly with polyserositis. Only bacteriological examination made it possible to establish a diagnosis of malignant listeriosis affecting the liver and lymph glands.

Group 6 was that of habitual abortion. Claims had been made by German and Czechoslovak workers that latent Listeria infection was responsible for habitual abortion, and that treatment by the appropriate antibiotics brought about a cure of the condition, resulting in normal deliveries. This had been based on serological observations made on over 50 cases of women who suffered from habitual abortion. However, these observations had been disputed but, since the claims had been made by reputable workers, he had included them in the types of listeriosis.

When they turned to the epidemiology of listeriosis, the picture became extremely complex. Cases in which the source was traced were very few and far between. One would expect that in Listeria of the newborn there would be ample evidence of infection in the mother, but this was rarely the case. There had been a number of cases where, following listeriosis in the newborn, it had been possible to isolate the organism from placenta, the lochia or the vagina.

Sloof in Holland in 1948 described the case of a 10-day-old infant who developed *Listeria meningitis*. During the last month of pregnancy, the mother suffered from conjunctivitis and a bloody purulent rhinitis. A few days after delivery, the mother died from meningitis. Although the C.S.F. showed Gram + bacilli, the cultures remained sterile, probably due to the chemotherapy which the mother received. There did not appear to be any doubt about the connection between these 2 cases.

In another case which occurred in the United States, the mother developed Listerial meningitis in the seventh month of pregnancy, and the newborn infant became ill 9 days after delivery and died of the same condition. But such cut and dried cases were few and far between, and it seemed that they must postulate a latent or subclinical infection in the mother during pregnancy.

Although infection was apparently widespread in the animal kingdom, it had been possible only in a few instances to incriminate infected animals. He had already mentioned the case of the Norwegian farmer who had died of meningitis acquired from his infected sheep. In 1958, Russian workers described an outbreak of listeriosis of the anginal type affecting 19 persons working in a collective farm. Pigs, supplied as food from a neighbouring farm, were suspected and on examination *L. monocytogenes* was isolated from the liver of one pig, and in the sera of 20 other pigs significant titres were obtained with Listeria antigens obtained from the patients and from the infected pig.

While few animals had actually been incriminated, many of the cases occurred in those persons having close contact with animals or with carcases and hides. Examples of these could be found in the Dutch farmer and veterinary surgeon and the Polish labourer whom he had already mentioned.

While no direct connection had been found between infected milk and human cases, there was evidence to suggest that the milk which was responsible for the outbreak described by Nyfeldt was infected with *L. monocytogenes*. It was also of interest to note that Potel observed that many of the mothers whose children died of granulomatosis infantiseptica drank raw cows' or goats' milk. The analogy with brucellosis was very obvious and, in view of the incidence of the disease in cattle, cow's milk as a source of infection must be taken into consideration.

Treatment of listeriosis had been revolutionised, like many other conditions, by the introduction of sulphonamides and antibiotics. Before the Fleming era, mortality was about 75 per cent. Since then, the death rate had fallen considerably. *In vitro* sensitivity tests revealed that the most active antibiotics in order of efficiency were erythromycin, magnamycin, oleandomycin, penicillin, Aureomycin† and Terramycin.†† However, they knew that the *in vitro* test did not necessarily correspond with the *in vivo* test. A substance active *in vitro* might have no results *in vivo*, because it was too quickly eliminated or did not even reach the infected tissue. This was particularly important in the case of bacteria like Listeria, which appeared rather rarely and for a brief period in the blood stream or in the body fluids, and which remained enclosed intracellularly in the reticuloendothelial system. *In vivo* tests with Listeria-infected mice showed that penicillin had a protective effect only when the antibiotic was given immediately after infection. It had no effect when it was given 2 hours after infection. Aureomycin, on the other hand, protected about 90 per cent. of the mice, even when given 24 hours after infection. Chloromycetin* was much less effective, protecting only 25 per cent. of the mice under the same conditions. On the whole, in mice, the tetracyclines were particularly good, while penicillin, streptomycin and Chloromycetin failed. Even with the tetracyclines, sterilisation of the infected organs was not obtained even though the mice survived. Bacteria were still present weeks after conclusion of treatment, in liver, spleen, urine and kidneys from the recovered mice.

In man bacitracin and polymyxin had no effect. Penicillin and streptomycin, when given alone, were also not particularly effective. Erythromycine had proved effective where other drugs had failed. Probably the most effective antibiotic was Aureomycin, since it, of all the antibiotics, passed most readily through the blood-brain barrier and the placental-foetal barrier. Sulphonamides in large doses had also proved effective. A combination of penicillin and sulphonamide apparently give quite good results. Treatment today usually consisted of the administration of sulphonamides together with two or three antibiotics, one of which was usually tetracycline.

The diagnosis of listeriosis rested with the bacteriologist, but the clinician must also be aware of the condition so that he could provide the bacteriologist with the necessary material. In the last few years, there had been a great increase in the number of cases reported. He did not think, however, that there had been an actual increase in the incidence of the disease, but rather that there had been an increased awareness on the part of the pathologist and, to a lesser extent, of the clinician.

In England there had been 9 cases of Listerial meningitis reported to date, the first of these in 1935, the second in 1939, and the remaining 7 in the last 3 years.

There was no doubt that in the past, and probably in many laboratories today, *L. monocytogenes* had been mistaken for the pneumococcus, a diphtheroid, a haemolytic streptococcus, *Haem. influenza* and even *Bact. coli*. In his own laboratory they had isolated an organism from a case of meningitis in an adult which was provisionally reported as a pneumococcus but, as they failed to confirm this with the usual tests, they investigated further and identified the organism as *L. monocytogenes*. In another of the British cases, the organism, which decolourised easily, was provisionally diagnosed as *Bact. coli*. Even as recently as 1954, a case of meningitis was reported in Britain as being due to a Corynebacterium. Last year he had been able to send the authors some specific sera, and they were able to identify the organism as *L. monocytogenes*. There was no doubt that in a busy laboratory the organism could be overlooked as a contaminating diphtheroid. Awareness was all. His own experience illustrated this. After he had isolated the organism from a case of adult meningitis in the Infectious Disease

*Chloramphenical †Chlortetracycline ††Oxytetracycline

Hospital, he discussed the case with a fellow pathologist in one of the Leicester hospitals. Six weeks later his colleague's chief technician isolated from the C.S.F. of another adult case of meningitis 3 or 4 colonies of an organism with the colonial and morphological features of a diphtheroid. The technician was prepared to discard these as contaminants, since the colonies had appeared only after 48 hours incubation. His colleague, however, recalled the conversation, and decided to investigate the organism further. As was to be expected, it turned out to be *L. monocytogenes*.

The diagnosis was important, since, if penicillin only was given, as it might well be, the treatment might be ineffective. This occurred in one of the cases published in this country. The organism was considered to be a diphtheroid, and the child was given penicillin. He appeared to recover, but a fortnight later relapsed, when the organism was this time identified as *L. monocytogenes*. Therapy was changed, but unfortunately the patient died.

The clinician, too, could play his part in unearthing many of these cases. A few weeks ago he had been talking to a friend in Italy who practised as a veterinary surgeon in Terni, a town not far from Rome. He had just attended a case of abortion in a cow. He suspected brucellosis, but nevertheless decided to send samples of foetal liver and spleen, and the placenta, to the Veterinary Department of the University of Perugia. All the specimens were found to be heavily infected with *L. monocytogenes*. The friend had asked him what the incubation period of Listeria meningitis was. He had told him that it was not definitely known. Now, he awaited the outcome with interest.

The General Discussion

The Chairman said that, to underline the interest shown in the paper, they might be interested to know that 24 hours before he left Bristol they had had a telephone call from a practitioner asking what pieces of a carcase of an animal they would require for diagnosis in a case in which he suspected listeriosis. Perhaps he could start the ball rolling by asking whether he was right in saying that in the past there had been a good deal of confusion between this disease and glandular fever in man.

Hypocalcaemia has been mentioned as one of the diseases from which listeriosis must be differentiated. Would the authors care to elaborate on this point a little? In view of the difficulty of using the occipito-atlantal space for intrathecal injections could the authors comment on the possibility of using a site, say in the lumbar region.

Dr. D. Luke (Belfast): "Is there not a risk that this whole subject of listeriosis in relation to public health is getting out of focus? I should hate to think that as a result of this paper the impression may be created, especially in the lay press, that there is any real danger of contracting listeriosis by drinking milk.

"In some of the cases quoted by Dr. Mair relating to the possible transfer of infection from animal to man the evidence is extremely tenuous and other possible sources of the infection have not been excluded.

"I would suggest, too, that the conditions in which the heat tolerance of *L. monocytogenes* was examined bear little resemblance to those obtaining in a modern pasteurising plant."

The Replies

Dr. N. S. Mair, in reply, said that the answer to the Chairman's first question was "Yes". The answer to the second question was that there were 3 possibilities. There was the possibility that it was a pure virus infection; it might be that it was Nyfeldt's *Listeria monocytogenes* that was causing the infection, and there was also the possibility of is being a combined infection.

It may well have been in Nyfeldt's case that the organism was dormant in the patient and was set alive by the virus.

Mr. A. D. Osborne said that hypocalcaemia had been included in the paper in a rather negative sense. In view of the relative frequency of the two conditions, there would naturally be a tendency to favour hypocalcaemia and, in general, he thought that the diagnosis would be much more likely. The effects of treatment would soon decide—he imagined in almost every case—which of the two conditions it was.

A point of importance was that in listeriosis there were almost invariably some signs of local paralysis of face muscles. That was the time to begin to think beyond the much more common condition of hypocalcaemia.

The site of injection and withdrawal of C.S.F. was a little dangerous. It might however be more satisfactory to inject the antibiotic as near to the site of the severe lesions as possible.

On the question of Erysipelas there should be no confusion. Morphologically, the organisms were somewhat dissimilar. The Listeria organism was of a beta-haemolytic type, whereas the other was of an alpha-haemolytic type. Motility in glucose broth was specific in its peculiar tumbling motion.

Mr. N. St. G. Hyslop in his reply, said that the question had been raised of the American workers who had recorded listeriosis in 2 employees in a poultry processing plant, and the possibility of Newcastle disease had been mentioned. The people concerned had developed quite a severe conjunctivitis and *L. monocytogenes* had been isolated. Whether this was a mixed infection with Newcastle disease, one could not say—although it seemed unlikely to him—but the possibility existed that this may have been yet another case of a virus disease potentiating a Listeria infection. Workers in Germany and elsewhere had recorded further cases of listeriosis among human beings engaged in handling poultry.

He had been asked about the survival rate in milk, and whether one should now look critically at pasteurisation temperatures. As far as he knew, no one had done any work on the "high temperature-short time" processes in relation to *L. monocytogenes*. Obviously, the effect of heat on all living organisms was a function of both time and temperature. If time was decreased as temperature increased the result might be very much the same as that achieved previously; and he believed that it was merely more economic from a commercial viewpoint to use a process which was a continuous one in which milk was trickled through a zone of high temperature. It was easier to do it that way and the method was no less effective against *Mycobacterium tuberculosis*, which was the principal organism against which pasteurisation was originally aimed. Whether "H.T.S.T." pasteurisation was more effective against Listeria than the low temperature method, they just did not know; but it was well known that thermoduric organisms may escape the effects of "H.T.S.T." pasteurisation and, unless the process was controlled carefully a quite wide spectrum of organisms may survive.

Speaking of his personal inclinations, he would certainly not drink milk from an animal affected with Listeriosis even if he knew that it had been pasteurised, and he felt that most of his audience would have to admit to being equally reluctant to do so.

A vote of thanks to the authors, and the opener of the discussion, was proposed by the **Chairman** and carried with acclamation, and the session then terminated.

Differential Diagnosis of some Conditions of the Canine and Feline Thorax

BY
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SUMMARY—Routine clinical examination of the chest is described.

The basic principles of viewing X-ray plates are mentioned.

The conditions of the canine and feline thorax dealt with are mostly those which can be confirmed radiologically during life.

The major points of differential diagnosis of each condition are dealt with within groups determined by some cardinal clinical sign. For convenience each condition is dealt with only under one group-heading, although it may have additional signs of some of the other groups.

The groups and conditions are as follows:

Gross Dyspnoea
- *Hydrothorax*
- *Pyothorax*
- *Pneumothorax*
- *Diaphragmatic hernia*
- *Pneumonia*

Cough
- *Distemper*
- *Tuberculosis*
- *Secondary lung tumour*
- *Filaroides osleri infestation of the trachea*
- *Kennel cough*
- *Feline influenza*

Tiring on exercise
- *Cardiac valvular disease*
- *Congenital cardiac disease*

Difficulty in swallowing
- *Oesophageal foreign body*
- *Feline oesophageal annular carcinoma*
- *Oesophageal diverticulum*
- *Mega-oesophagus (Achalasia)*
- *Oesophageal dilatation associated with vascular anomalies of the right aortic arch.*

Introduction

In dealing with this subject emphasis is placed on those conditions which at certain stages of their development or in certain forms have a diagnostic radiological appearance.

As an immediate radiological interpretation is not available to everyone and diagnosis can sometimes be confirmed by other methods, these will now be considered.

PART I

Methods of Diagnosis other than Radiological

History

This is sometimes of great value, e.g. of a road accident, in the case of diaphragmatic hernia; known infection of the household in tuberculosis; the presence, or previous removal of tumour in secondary lung tumour; and the origin from an infected kennel of a suspected case of *Filaroides osleri* infestation. In the latter disease the owner's description of the type of cough is sometimes pathognomonic.

Examination

This description is restricted to the chest, it being assumed that other parts and systems will be examined also as a routine.

Inspection:

Respiration may be classed as 'dyspnoeic' when there is obvious difficulty, associated with excessive abdominal movement and possibly opening of the mouth, outstretched head and nostril movement. The worst cases maintain a standing or sitting posture. Thoracic movement may have a definite pattern within each respiratory cycle.

Respiratory rate:

The significance of respiratory rate alone may be difficult to assess, as it can vary so much with excitement, size of animal, previous exertion, environment, fear, pain, etc. It is advisable to count the rate by inspection of chest movement for 30 to 60 seconds before handling, and if the animal has been at rest, any rate over 30 per minute is usually significantly high and tends to increase proportionately to the amount of occlusion of the lung field. Daily rates taken under comparable conditions are a useful indication of progress.

Percussion:

This is preferably performed in a silent room, where the acoustics are familiar to the percussor; with the patient in the standing position on a low table. A finger or piece of wood such as a ruler is applied closely to the chest wall, usually in the space between two ribs, and struck sharply by a plectrum or finger tip. Routine procedure is desirable; a suitable one is to start anteriorly and dorsally on one side and move downwards. The examination is repeated on the other side, and if no discrepancy is found, it is restarted dorsally on the original side, one or two rib spaces further back. This examination should cover the area of the thoracic wall which contains the lung field, the boundaries of which are limited by the pectoral limb anteriorly; the spinal muscle mass above; the line of diaphragmatic attachment posteriorly and the sternum below. Normal resonance is less over the heart and in particularly heavily-muscled or obese animals.

Auscultation:

A chestpiece diaphragm is thought to be an advantage in detecting sounds. Avoidance of all hair crackling is impossible. Systematic coverage and comparison of left and right sides is carried out as in per-

cussion. More attention should be given to recognition of grosser area discrepancies than identification of adventitious sounds in a small area. Complete lack of sound associated with a gross pulmonary lesion is a more significant abnormality than the presence of adventitious sounds. The latter can be classified as: rhonchi—(dry sounds)—sonorous or sibilant; and rhales—(moist sounds)—also sonorous and sibilant. Within that classification can be great variation and it is very difficult to associate any single sound with a specific lesion. One loud focus may transmit over a large area. Pleuritic rubs are rarely, if ever, present or identifiable.

Thoracentesis

This is usually performed very low on the chest wall, between the 7th and 8th costal cartilages, with syringe and a one inch, eighteen gauge hypodermic needle, without local anaesthesia. Removal of large amounts of fluid usually requires a larger needle.

Examination of oesophagus and trachea.

Abnormalities may be visualised by the use of endoscopic apparatus. Passage of a sound, or the meat test may indicate oesophageal obstruction.

Factors in Radiological Examination

The interpretation of radiographs of the chest of the dog and cat has been the subject of very considerable advance in recent years and is constantly developing.

The features which can be visualised are the result of pathological processes which either alter the shape or size of the structures of the thorax; or cause a change in the radio-opacity and so alter the contrast between related structures.

The radio-opacity of the body tissues may be listed as follows:

(1) Bone (and calcified structures) most dense, (lightest shade on the X-ray plate)
(2) Fluid (blood vessels and blood filled organs)
(3) Soft tissues (muscle, etc.)
(4) Fat
(5) Gas (and gas filled organs)—least dense (darkest on the plate)

Thus in the thorax the heart shadow is clearly seen because it is a large, fluid-filled organ surrounded by the gas-containing lungs. The pulmonary blood vessels are also visible, especially where they are of moderate size; whereas the bronchi, except for the proximal portions, are not visible because, like lungs, they are gas filled.

This effect of the relative radio-opacity of the background and the structures may be illustrated by radiographing a box which has a number of straws passing through it from side to side in both directions. If one set of straws is left empty (air-filled) they may be taken to represent bronchi; and if the others are water filled they may be taken to represent pulmonary vessels. (See Fig. 1.)

When the box contains air (equivalent to normal gas filled lung) the air filled straws (bronchi) are not visualised but the water filled straws (vessels) stand out clearly.

If the background is now altered by filling the box with water—to represent the alteration which occurs in pneumonia—the air-filled straws (bronchi) now stand out clearly and the water-filled straws (vessels) become invisible.

This appreciation of visibility due to alteration in contrast is a basic factor in radiographic interpretation and is as important as the recognition of alteration in organ size and shape.

It is possible to consider the "lung field" as consisting of 3 zones, for the purposes of radiographic examination.

(1) *Hilar Zone:* Is the inner one third, closest to the bifurcation of the bronchus.

At this level the pulmonary vessels are largest in size and most readily seen. The lung tissue here is partially obscured by the vessels. The bronchi are large and are visible because they are flanked by the pulmonary vessels.

(2) *The Central Zone:* Is the middle one third and here the pulmonary vessels are smaller, but still visible. The bronchi are no longer readily discernible in the normal lung.

(3) *The Peripheral Zone:* Is the outer one third, and here the pulmonary vessels are too small to be identified and the lung field should exhibit an even radio-translucency.

PART II

On presentation our patients can be loosely grouped according to history or some cardinal clinical sign. We have chosen this form of classification as it seems the usual way in which diagnosis is approached in practice. Although a single disease is dealt with under only one group-heading this is done for convenience, and some cases may well have in addition features of other groups, while other individuals need not necessarily show the cardinal sign of their group.

Group I. Gross Dyspnoea

(a) Hydrothorax (b) Pyothorax (c) Pneumothorax (d) Diaphragmatic Hernia (e) Pneumonia

(a) "Hydrothorax"

The authors use this term somewhat loosely to indicate the presence of fluid, not frankly purulent, in the pleural cavities. It is not a specific condition and is usually associated with other disease processes:

1. Pleurisy with effusion. This is most commonly associated with tuberculosis infection, but other organisms are occasionally responsible.
2. Cardiac failure, primary or secondary.
3. Lymphatic blockage, associated with primary neoplasm of lymph node, or less often secondary neoplasm or inflammatory change.

Incidence: Older age groups are more likely to be affected.

History: The dyspnoea usually occurs fairly rapidly, over a period of a few days, though other signs of illness may have been of longer duration.

Clinical Examination: The respiratory rate is rapid. Percussion in the standing position can usually determine a horizontal "fluid line" with decreased resonance below. On turning the animal on its back the sternal region becomes resonant; this procedure, however, is often distressing to the animal and may be dangerous.

Difficulty in determining such a line arises when

there is too little fluid, or when the pleural cavities are so full that there is hardly any resonant area.

Auscultation reveals lack of sound below the "fluid line". Thoracentesis reveals the nature of the fluid. The needle is readily blocked by muscle, lung or fibrin, and the first puncture may be unsuccessful. It is usual for the fluid to be similarly dispersed in right and left sides. Small amounts of fluid, detectable radiologically, can not be determined clinically.

X-Ray: The presence of free fluid in the pleural cavity causes loss of visibility of the various structures of the thorax in a degree which depends on the actual amount of fluid present. Small amounts of fluid will separate the lung lobes from the thoracic wall and from each other and the fluid will appear dense.

Greater amounts will cause a nearly homogoneous obscurity of detail and will merge in with the shadow of the liver (which normally points the diaphragmatic line).

If the lateral radiographs are taken with the dog standing, the fluid level will be seen, and it can be altered relative to the thoracic wall by causing the dog to change position. (Fig. 2.)

Diagnosis: The underlying cause or compound causes resulting in hydrothorax may be difficult to determine. Tuberculosis is dealt with later. Primary cardiac incompetence may be diagnosed on auscultation, and this and secondary heart failure may be associated with subcutaneous oedema, engorged jugulars and ascites. Lymph node involvement may be recognised radiologically if the amount of pleural fluid is not excessive, and tumour may be found in other nodes or other tissues.

Prognosis: In this group mostly hopeless. One known case resolved spontaneously and the dog lived 6 months.

Treatment: This is rarely undertaken. It is inadvisable in tuberculosis. Where there seems hope, drainage, oxygen, heart stimulants and antibiotics can be used.

The cat does not seem to be affected with hydrothorax nearly so often as the dog, and in this species tuberculous pleurisy is uncommon.

(b) Pyothorax

This is dealt with separately because the cat suffers from this condition more often than hydrothorax. It is usually of rapid onset and too often the patient is presented in a collapsed condition. Mucous membranes are often very pale, as in feline enteritis, but abdominal pain is not obvious and there is less tendency to vomit. Nocardia species have been cultured from some cases.

Diagnosis as for hydrothorax. Laying the animal on its back is highly dangerous, and anaesthesia a grave risk.

X-Ray: Gives a picture indistinguishable from hydrothorax.

Treatment: As for hydrothorax. In the dog, it may be associated with penetrating oesophageal foreign body.

(c) Pneumothorax

This is a fairly uncommon condition in either dog or cat. It usually follows a physical accident, but is sometimes associated with the breakdown of an inflammatory or neoplastic lung lesion underlying and involving the pleura.

Percussion: Any possible hyper-resonance is difficult to detect.

X-Ray: Shows the same features of separation of the lung lobes from the chest wall as in hydrothorax, but here the space between the lungs and the thoracic wall is less dense than the lung tissue and so the lobe edges show up clearly. Correspondingly the various structures of the mediastinum are more clearly visualised than normal; this effect is especially noticeable between the heart and the sternum. (Fig. 3.)

This condition may be associated with a subcutaneous emphysema, when gas will be seen between the muscles and under the skin.

Treatment: Aspirate the air from the pleural cavities employing a "water-drain" with the additional use of negative pressure in the bottle when the condition is of severe degree. In many cases of traumatic origin recovery is spontaneous.

Prognosis: Generally good.

(d) Diaphragmatic Hernia

Incidence: Not uncommon.

History: There is usually some previous injury, usually a road accident. Most cases are presented soon afterwards, but the odd animal may not be brought for some months. Occasionally the condition may be congenital.

Signs: Dyspnoea and occasional vomiting.

Percussion: There is usually a loss of resonance ventrally in one diaphragmatic zone. It is more likely to be asymmetrical between right and left sides than in a case of hydrothorax, but may be complicated by the presence of fluid.

Auscultation: There is some loss of lung or heart sound, depending on the amount and position of herniated tissues.

On abdominal palpation a lack of abdominal contents may be recognised, but this is much less readily detected in the cat than in the dog.

X-Ray: The major feature is loss of the diaphragmatic line, which is normally a somewhat "compound" line since it is representing a "domed-shape" on a plane film. The detail of the heart shadow is frequently obscured. A dorso-ventral (D.V.) view will reveal which side of the thorax is affected.

The appearance will vary according to the abdominal contents which have been herniated:

(1) If the stomach is herniated, repeat films may show an alteration in the contained gas shadow.

(2) Where one or two liver lobes are herniated through a small tear the case may be complicated by the development of a hydrothorax as a result of passive congestion of the herniated part of the liver causing massive exudation from the serous surface. (Fig. 4.)

(3) Only if the alimentary canal has been herniated, is barium meal administration helpful and in certain cases (intra-pericardial—diaphragmatic hernia) it may be the only method available of reaching a diagnosis. (Fig. 5.)

Cholecystography may be of help, but in a limited number of cases in Glasgow has not proved of value.

Treatment: Surgical reduction and correction of the

diaphragmatic tear.
Prognosis: Fairly good. It depends on the degree of shock and adhesions, etc.

(e) Pneumonia

This occasionally causes a severe dyspnoea. It may be associated with cardiac failure or infection such as toxoplasmosis complicating distemper. A few cases of a severe proliferative pneumonia of the dog have been seen.
X-Ray: (Olsson, 1952). The pulmonary vessels are rendered invisible by the pathological changes in the lung tissue. The bronchi are prominent unless fluid-filled. In cases which exhibit lobar distribution the affected lobe shows increased density and its borders can be clearly seen. Cases which have a widespread distribution of lung changes show an overall patchy loss of translucency.

The localisation of this lesion can be accurately carried out and the progress of the case observed. (Fig. 6a and b.)

Group II. Cough

(a) Distemper (b) Kennel cough (c) Tuberculosis
(d) Secondary lung tumour
(e) *Filaroides osleri* infestation
(f) Feline influenza

(a) Distemper

Cough is a very common sign, soft initially, but soon hard and the owner may then suggest that there is a "bone in the throat".
Diagnosis: This depends very largely on history of a known source of infection; an age incidence usually under one year; and on the presence of other signs such as inflamed or catarrhal mucosae, diarrhoea, pad hyperkeratinisation and nervous complications. No absolutely specific test is yet available.

Pneumonia of a moderate degree is common in the earlier part of the disease, with increased respiratory rate and blowing sounds on auscultation. Rhales and rhonchi are less common but may occur especially with heavy secondary bacterial infection.
X-Ray: This condition includes in most instances a virus pneumonia with no associated consolidation and so it presents only very slight features radiographically. The lungs exhibit a fairly uniform degree of excessive fluid pathologically and this is represented radiographically as a slight decrease in translucency throughout the lung-field with an additional 'patchy' loss of translucency at the areas where the lungs are most affected.

(b) "Kennel Cough"

This is a syndrome, not an entity. Some cases termed kennel cough are caused by distemper, but there are probably other respiratory infections which result in a marked cough without the animal being systemically very ill, and in an individual the cough may persist for many weeks. There appears to be an infectious disease which can cause an outbreak of coughing in kennelled dogs probably immune to distemper.
Diagnosis: Is based on a history of contact with an infected kennel and lack of development of the signs of distemper.

(c) Tuberculosis

Cause: Tubercle bacilli, nearly always of human type in Britain, sometimes of bovine type and very rarely avian (Francis, 1958).
Incidence: Present-day figures for Britain are unknown. In Glasgow it is estimated that of every 500 dogs presented at our Clinic for any reason, at least one will have tuberculosis confirmed. It is uncommon in animals under 1 year of age, but has been recorded by us at all ages from 5 months to 15 years.
History: Signs have usually been noticed for 2-3 months, sometimes longer. If it is suggested that the dog almost certainly has had some contact with human infection, the owner may volunteer such information.
Signs: The signs of this disease are very variable, according to the sites affected.

The thoracic organs are the most commonly involved but are not necessarily associated with diagnostic clinical signs.

The most common lesion is formative tubercle of the bronchial lymph nodes.
Auscultation and percussion: These may reveal dull areas due to pleural fluid or formative tubercle of the lung, and rarely increased resonance may be recognised where bronchiectasis is present. Adventitious sounds are not common. Though pericarditis is a common lesion, effusion into the pericardial sac does not seem to be associated with splashing sounds.

In previous years tuberculosis was mainly diagnosed during life only when advanced, with pleural effusion and wasting. Now earlier changes can be recognised radiologically, and many of our cases have been diagnosed by routine chest X-ray of dogs in which tuberculosis was suspected.

The main reasons for suspicion may be listed, approximately in order of frequency with which they occur, as follows:

(a) Pyrexia, commonly fluctuating with normal temperature, of long duration.
(b) Respiratory signs of coughing, pleurisy, and accelerated rate.
(c) Wasting.
(d) Abdominal signs. Enlarged and sometimes painful liver. Enlargement may be associated with amyloid change. Distension of the abdomen by fluid may occur secondary to ascites or tuberculous peritonitis. Vomiting and diarrhoea are not rare and the latter occasionally contains blood.
(e) A very high urine protein. The precipitate on contact of urine and sulphosalicylic acid may form a white gel, while quantitatively levels well over 200 mgm./100 ml. may be found. This may be associated with amyloid change of the kidney, which is usually secondary to tuberculosis.
(f) Less common but not unimportant findings are:— Lymph node enlargement. Indolent ulcer. Oedema, which may be associated with amyloidosis or lymph blockage. Marie's disease.

X-Ray: (Olsson, 1957). Tuberculosis may exhibit exudative features in which case it is radiographically a hydrothorax and must be identified by other means. It is most often of the 'formative' type and affects especially the lymphatic tissues. The bronchial lymph nodes are most often affected and exhibit either moderate or gross enlargement. (Fig 7.)

At the early stage the reactive lymphatic tissue may appear to form a 'cuff' round the distal end of the trachea. At this stage and later the point of the bifurcation of the bronchus often appears to be 'pinched'.

'Secondary' reactions may be found in the anterior sternal lymph nodes, which can be detected bulging up and separating the anterior free edges of the apical lobes. The small patches of lymphatic tissue in the posterior mediastinal pleura may also be affected and will be seen to be not only thickened, but nodular and roughened as well.

Occasionally a bronchial lymph node may become necrotic and burst out into the 'intermediate space' and the entire area of the intermediate lobe will become filled with exudative fluid which is contained within thickened reactive pleural walls. This gives the appearance of a 'second heart' placed somewhat further back and higher than the normal, in the chest. (Fig. 8.)

Pulmonary cavitation is relatively uncommon and may occupy a central or a peripheral situation. (Fig. 9.)

Calcified nodules may occur on the pleura and tend to occur in groups which appear somewhat like a small group of rice grains lying together on the pleura, or as slightly larger more discrete masses on the diaphragmatic pleura.

Confirmation of Diagnosis: Some radiographic appearances are diagnostic. Microscopical examination of thoracic exudate frequently shows acid-fact bacilli, but repeated examination may be necessary. Dropsical fluid, urine or a swab from a skin ulcer or opened abscess may also be positive microscopically. Culture of these fluids is a more sensitive method of demonstrating the organism.

Culture of saline-moistened pharyngeal and rectal swabs or P.M. tissues swiftly delivered to an experienced laboratory is frequently successful and typing by animal inoculation of culture is to be strongly recommended.

A human with human or bovine type infection, or milk, probably with bovine type bacteria has probably been responsible for the dog's infection, and may be traced. The human is probably in need of treatment. (Hawthorne, 1957.)

Histology of biopsy or p.m. material is diagnostic

Tuberculin test. This has not proved reliable in the dog, false negatives having been common. (Thorpe, 1957.)

B.C.G. Live B.C.G. vaccine given intradermally has been experimented with and seems more useful than tuberculin, but the advanced case may not react, positive reaction being marked swelling at the injection site and ulceration within 14 days. The interpretation of this reaction is not yet fully known.

Cat. In this species the main lesions are abdominal and usually due to bovine-type bacilli from milk. Chest and other lesions may occur. In recent years the incidence has fallen markedly in our experience.

(d) Secondary Lung Tumour

This occurs not uncommonly, especially as a sequel to mammary adenocarcinoma and is therefore found especially in ageing bitches.

A mammary tumour, which may be quite small, may be present or there is a history of previous surgical removal.

Signs: Cough, weakness and breathlessness. Auscultation and percussion are not of great value because the lesions are usually discrete and scattered widely throughout the lung. Often the owners may notice little or no change until the condition is far advanced.

X-Ray: Confirms diagnosis; most cases show "cannonball" secondaries, i.e., circular masses of neoplasm scattered through the lung field. These are of varying size in most cases since the metastases do not all form at one time. (Fig. 10.)

Very rarely the spread of tumour to the lungs takes place very rapidly and then the reaction is not unlike an overall consolidation "rubber lungs". These cases show rapidly developed, marked respiratory distress.

(e) *Filaroides osleri* Infestation (Urquhart, Jarrett and O'Sullivan, 1954. Olsen, Wilford and Bracken, 1959).

The mode of transmission of this tracheal worm is not known. There may be an intermediate host.

We have come across 21 cases in the past 2 years, mostly associated with 2 premises and the real incidence is unknown in this country. It is a condition which may well have been overlooked. Some dogs may show no sign whatever, yet pass larvae in their faeces, and have nodules in their tracheas, composed of a mass of worms below the lining epithelium. Others are severely affected and the usual history is that at a few months of age, or sometimes not till 1 year old, a dog is noticed to have an expiratory wheezing attack on exercise or on becoming excited. At rest or with moderate exercise the animal may appear normal. Milder cases may have occasional attacks for a very long time, but severe cases have many every day, starting a spasm which may end with cyanosis of the tongue and collapse, followed by partial recovery in a few minutes. Such cases lose weight and are either destroyed or may develop pneumonia and die, though the worm itself does not appear to cause lung damage.

Pinching the trachea makes most normal dogs cough 2-4 times but one that has Filaroides will probably start a wheezing spasm. This sounds different from other coughs and sometimes ends with the ejection of a little fluid or mucus. Expiration is prolonged and forced, with head and neck extended, back arched and abdomen contracted.

Auscultation often reveals sonorous and sibilant rhonchi while percussion is usually normal.

X-Ray: The feature which is recognisable radiographically is the projection upward into the lumen of the trachea close to the point of bifurcation, of the worm-containing nodules which develop in this disease. N.B. The point where the apical bronchus crosses the lower edge of the trachea should *not* be mistaken for a nodule and if this is the only part showing such a feature a negative diagnosis should be given. (Fig. 11.)

An alternative method of diagnosis is the demonstration of larvae in the faeces. This can sometimes be done by mixing a small speck of faeces with a large drop of water and recognising slightly motile larvae microscopically, but concentration techniques using

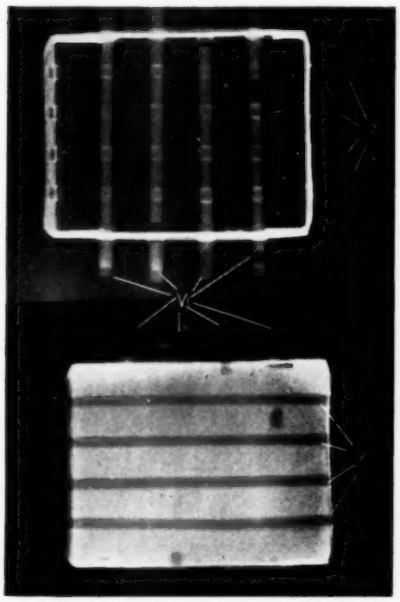

FIG. 1.—Radiograph of model to illustrate how pulmonary consolidation alters the normal contrast in the lung. A box is used to represent the lung. Two sets of straws pass across the box: air-filled straws pass transversely and represent bronchi (B); water-filled straws pass from top to bottom and represent pulmonary vessels (V).

Top: The box is air filled to represent normal lung. The "bronchi" are invisible because, like the lung, they are air filled. The "pulmonary vessels" are fluid filled and stand out clearly because of their contrast with the background.

Bottom: The box is water filled to represent consolidated (pneumonic) lung. The structure visibility is now reversed because of the change in contrast. Thus the "bronchi" are seen against the fluid background and the "pulmonary vessels" are invisible.

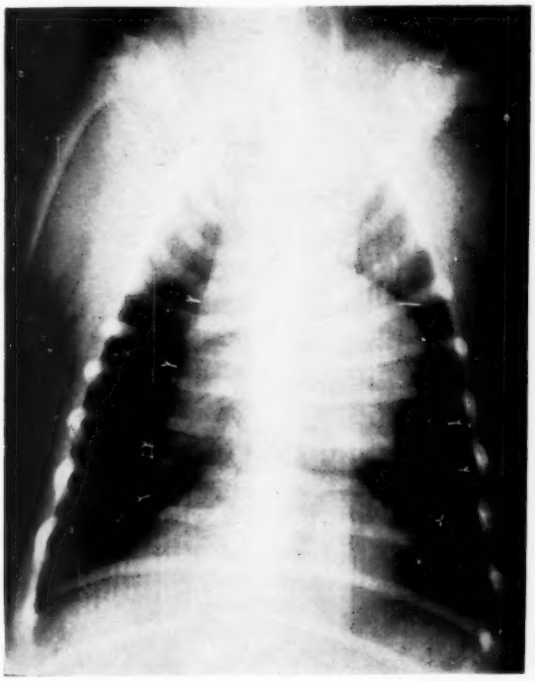

FIG. 3.—Pneumothorax. The lung borders (Y) are separated from the thoracic wall and are clearly seen because of the contrast afforded by the gas in the pleural cavity.

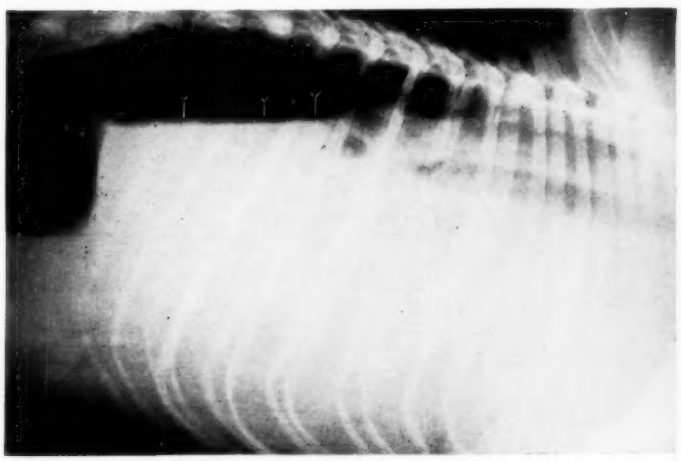

FIG. 2.—Hydrothorax. Fluid free in the pleural cavity obscures the thoracic structures because of its density. The fluid level (Y) cannot be determined if the dog is lying on its side.

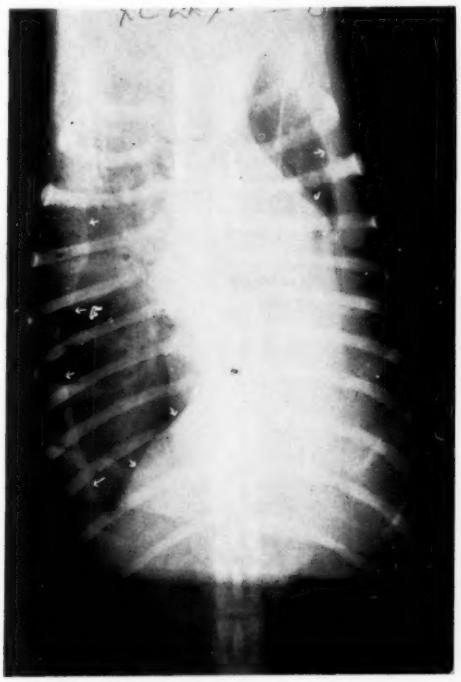

FIG. 4.—Diaphragmatic hernia with hydrothorax. A slight degree of hydrothorax has developed subsequent to herniation of part of the liver. The lung lobes are separated from the thoracic wall (↓) and at the right cardiac-diaphragmatic lobe fissure the lobes are separated from each other (↑ F).

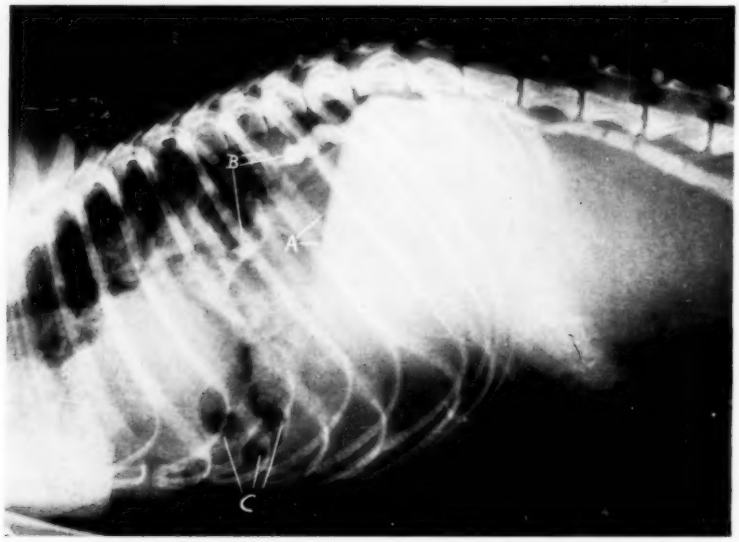

FIG. 5.—Diaphragmatic hernia. The liver is undisplaced and so the diaphragmatic line shows little abnormality (A). The barium (B) and gas (C) filled intestine can be seen obscuring the normal thoracic picture.

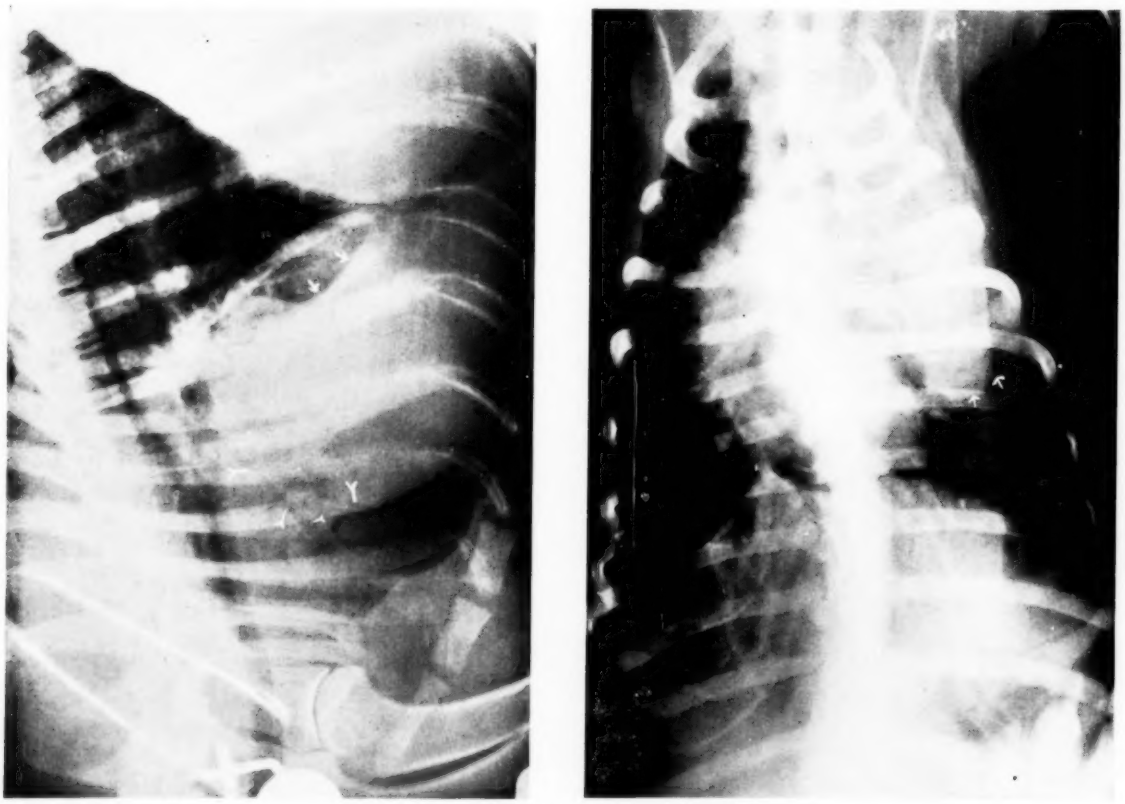

FIG. 6.—Pulmonary consolidation. The right apical and cardiac lung lobes are consolidated. The bronchi can be seen traversing the affected lobes (⅄). The posterior border of the cardiac lobe can be seen. (↑).

FIG. 7.—Tuberculosis of the bronchial lymph nodes. The lymph nodes are markedly enlarged due to "formative" reaction (↑). The distal end of the trachea is displaced downwards and is slightly compressed. (C).

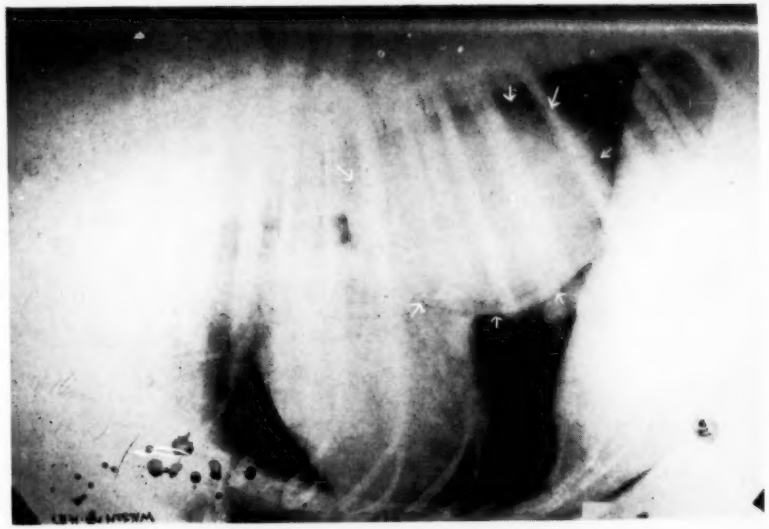

FIG. 8.—Posterior mediastinal "blow-out". Necrosis of a tuberculous lymph node causes infection in the posterior mediastinum and fluid collects between the reactive thickened pleurae (↑).

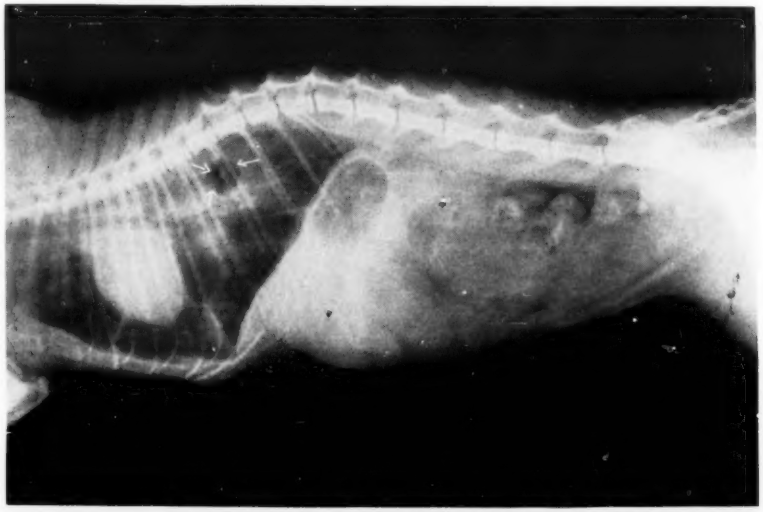

FIG. 9.—Tuberculous pulmonary cavitation (cat). There is a rather regular cavity (↑) in the upper central position of the diaphragmatic lobe.

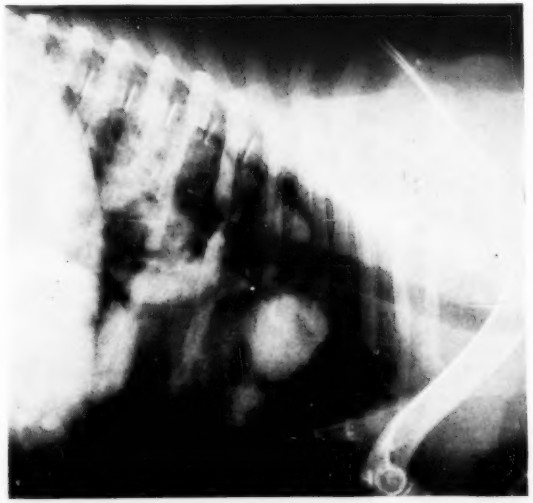

FIG. 10.—Secondary tumour in lung. "Cannon-ball" circular tumours, varying in size according to time of duration, occur scattered throughout the lung substance.

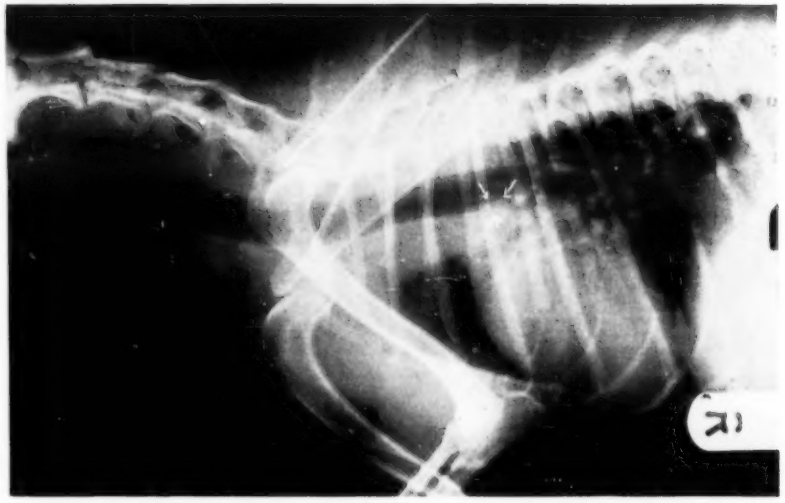

FIG. 11.—*Filaroides osleri*. *F. Osleri* infestation produces nodules in the trachea, close to the bifurcation (↑). The nodules can be seen projecting up into the lumen of the trachea, contrasted against the contained gas.

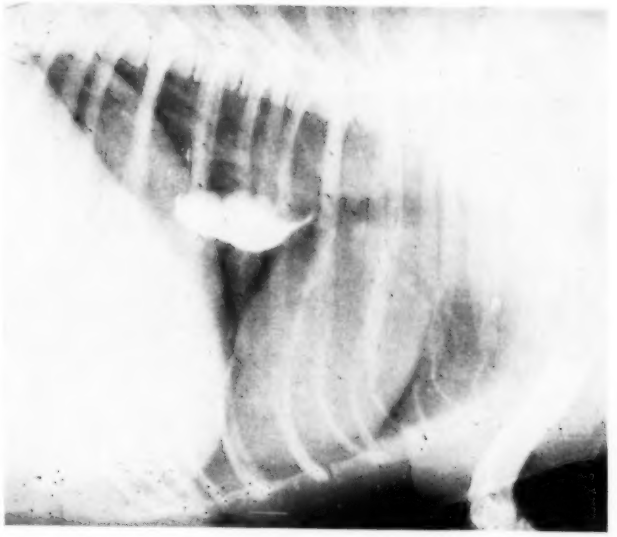

FIG. 12.—Oesophageal diverticulum. The diverticulum retains barium cream after the rest of the meal has passed on.

FIG. 13.—Megaoesophagus (cardiospasm). The dilated oesophagus is filled with barium cream and retained food. The distal end of the barium shadow is rough because of the "mass of food" which lies anterior to the diaphragm.

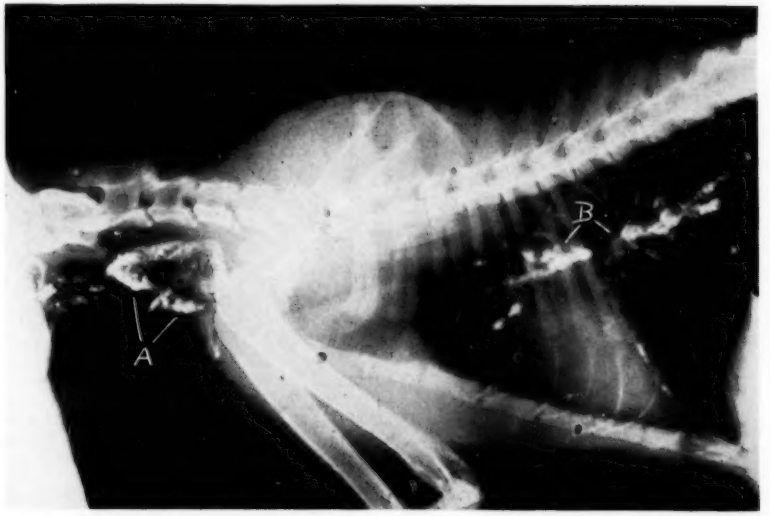

FIG. 14.—Feline oesophageal carcinoma. The barium cream passes into the ulcerated tumour mass and outlines it (A). Some barium has passed down into the bronchi due to aspiration (B).

zinc sulphate or a Baermann apparatus are more delicate and often give a positive result.

The nodules may be seen on bronchoscopic examination.

No treatment has been found of value, but some animals make a spontaneous clinical recovery after many months, though those followed up for 1 year have continued to pass larvae in their faeces.

Disposal of faeces to avoid infection is advocated. Pesticides may eliminate the possible intermediate host.

The history of a young dog having wheezing attacks for some months, without other symptoms, is not known to occur in any other condition.

The older dog may be more resistant to infection. There is presumptive evidence that one breeding premises brought infection in 4 years ago. Recently four 7-year-old home-bred bitches were destroyed without even having shown signs of illness, and 3 of them had small nodules in their tracheas.

(f) Feline Influenza

Cause: two viruses have been identified in cats showing the syndrome of this disease, or two syndromes which are not clearly distinguishable on clinical examination. Baker (1944) described one virus and Gledhill (1952) the other.

Cat influenza is the commonest known cause of coughing in this species. It is almost invariably accompanied by sneezing and some catarrh of eyes and nose. Ulceration of the tongue is less common. Some degree of pneumonia often occurs, but this is severe only in a minority of cases and the death rate is low. Younger animals are the most likely to develop severe infections. Diagnosis is usually comparatively easy on clinical signs and known infection in a district or cattery. Baker's virus is susceptible to sulphonamides and antibiotics. Catteries with individual accommodation having free access to the open air are less likely to have a severe epidemic of this infection.

Group III. Tiring On Exercise

Cardiac Valvular Disease

Under this heading is discussed only the syndrome, recognised in older dogs, of tiring and panting on exercise, which at its most extreme results in fainting and cyanosis. A persistent cough is common and may be the presenting feature.

On auscultation, the earliest detectable abnormality is that the termination of the second heart sound becomes less sharply defined, then it becomes muffled in a murmur, and latterly first and second sounds cannot be distinguished from each other and from a gross murmur. In an advanced stage there may be visible excess cardiac movement on the chest wall, and palpation of this area confirms the excessive movement and an irregular and high heart rate. Recognition of an enlarged dull cardiac area on percussion is considered difficult. Terminally there come the signs of heart failure—engorged jugular veins, big liver, ascites, subcutaneous edema and possibly hydrothorax. Some dogs can live a long time with a gross murmur even though showing fainting attacks from time to time. These are not always true faints, as consciousness is sometimes retained and there may be muscular spasm during the attack.

The valvular dysfunction may possibly be due to a primary valve lesion associated with some infection, or of unknown origin; it may be associated with cardiac dilatation secondary to circulatory disturbance in other organs, such as chronic nephritis.

Congenital Cardiac Disease

The incidence in this country is unknown. In Glasgow 4 young Boxers have been brought for attention as they seemed to tire readily on exercise and also showed some panting.

Auscultation revealed a very gross murmur in each case and X-Ray showed a grossly enlarged heart, a prominent engorged posterior vena cava and a very poorly defined aortic shadow—which sometimes was completely invisible.

In two dogs angicardiograms showed gross retention of the dye in the left heart, indicating the presence of a marked sub-aortic stenosis. These findings were confirmed at autopsy.

Prognosis: Hopeless.

Group IV. Difficulty in Swallowing

Oesophageal Foreign Body

Incidence: Quite common.

History: Sudden onset; vomiting of saliva-covered undigested food, after having eaten bones. Other food or objects rarely cause obstruction at this site.

Signs: In the case of complete obstruction, vomiting will be persistent for a few days after eating solid food. Later there may be no vomiting because of anorexia. There will be only a slight tendency to retch without eating. Liquids are less likely to be vomited. Incomplete obstruction may be caused by flat foreign bodies such as the proximal epiphysis of the pig's tibia or a sliver of scapula, which by changing position slightly may, on some occasions, permit degluttition, while on others there is complete obstruction. Perforation of the oesophageal wall is a major hazard, and may occur if a foreign body with a sizeable projection is present for one week or more. This is liable to be followed by a mediastinal infection and septicaemia; often fatal.

Diagnosis: The history is often indicative. The administration of a piece of meat or other solid material is usually followed by vomiting within a few minutes.

A sound or oesophagoscope, when passed in an anaesthetised animal will indicate the site of the obstruction. Froth often obscures vision through the oesophagoscope and must be aspirated.

X-Ray: Commonly a bony obstruction is seen on lateral projection, in the posterior mediastinal oesophagus. Rarely the foreign body is not dense radiologically, e.g., if largely cartilaginous, in which case it can be delineated by the administration of barium.

Treatment: Many foreign bodies can be removed by forceps and oesophagoscope, but this manipulation must be done very gently otherwise considerable damage may be caused. When the obstruction is not readily moveable trans-thoracic oesophagotomy should be performed.

Oesophageal Diverticulum

Incidence: This occurs very rarely, following relief

FIG. 14.—Feline oesophageal carcinoma. The barium cream passes into the ulcerated tumour mass and outlines it (A). Some barium has passed down into the bronchi due to aspiration (B).

of oesophageal obstruction. The diverticulum is a pouch of mucous membrane which has passed through a defect in the muscular wall.
Signs: There is a tendency to retch and vomit after eating solids.
Diagnosis: The condition is strongly suspected from the history. It is confirmed by administering a barium meal, which leaves a residue in the diverticulum, visible on radiological examination. (Fig. 12.)
Treatment: Surgical reduction following thoracotomy.
Prognosis: Good.

Mega-Oesophagus (Synonyms: Cardiospasm, Achalasia)

This condition is thought to be the result of contraction of the cardiac sphincter due to shortening of the circular muscle fibres associated with dysfunction of the local nerve plexuses. It has been produced experimentally in cats. (Deloyers, Cordier and Duprey, 1957).
Incidence: Uncommon, but it may occur more often in certain breed of dogs.

Two distinct age groups are affected:
(a) Immature animals.
(b) Middle-aged or older.

The rate of onset is not well known. There is fairly frequent vomiting of saliva-covered solid food soon after eating. This may be re-eaten and retained.
Diagnosis: Confirmation depends on radiological demonstration of a grossly distended oesophagus, following administration of a barium meal. This distension is more or less regular and extends backwards to the diaphragm. The posterior end of the oesophageal shadow may be roughened by the presence of retained food. (Fig. 13.)
Treatment, Prognosis: Some good results have been obtained by a plastic operation, performed through the thorax, designed to destroy the functional efficiency of the cardiac sphincter.

Vascular Anomalies (Detweiler and Allan, 1955; Lawson, Penhale and Smith, 1957).
Incidence: Uncommon. Pups are usually presented within a short time of starting to eat solid food, as the condition is congenital.
Cause: Developmental anomalies of the right aortic arch, with the development of a "vascular ring" causing oesophageal compression.
Signs: Vomiting of saliva-covered food soon after eating. Frequent attempts to re-eat are made, but on each occasion the food is again vomited until the animal retires exhausted. The cervical oesophagus becomes visibly distended on eating.
Diagnosis: Age-incidence, history, plus x-ray with a barium meal which shows a distension of the oesophagus anterior to the base of the heart.
(See *Vet Rec.*, **69**, 326)
Treatment: Excision of some part of the constricting vascular ring if possible.
Prognosis: Poor, as the animals are usually grossly under-nourished, and under-developed when first seen, and some secondary dilatation of the oesophagus will persist.

Annular Oesophageal Carcinoma of the Cat
Incidence: Uncommon. Older cats.
Signs: When presented the condition has usually been in existence for some weeks at least; and the animal is in very poor condition. The predominant sign is anorexia, inability to swallow, though vomiting after food may have occurred at an earlier stage.
Diagnosis: Usually indicated by history. It is confirmed by X-Ray after a small dose of barium which is unlikely to pass the obstruction, and usually infiltrates the roughened surface of the tumour. (Fig 14.)
Prognosis: Hopeless.

Acknowledgements. We wish to thank Professor W. L. Weipers for his interest and Mr. W. B. Martin for his clinical work. Many colleagues have assisted in the confirmation of diagnoses.

References

BAKER, J. A. (1944). *Jl. exp. Med.*, **79**, 159.
CORDIER, R., DUPREY, H., and DELOYERS, L. (1957). *Annals of Surgery*, **146**, 167.
DETWEILER, D. K., and ALLAN, M. W. (1955). *Cornell Vet.*, **45**, 209.
FRANCIS, JOHN. (1958). *"Tuberculosis in Animals & Man"*, Cassell, London.
GLEDHILL, A. W. (1952). *Vet. Rec.*, **64**, 723.
HAWTHORNE, V. M. *et al.* (1957). *British Med. J.*, No. 5046.
LAWSON, D. D., PENHALE, B., and SMITH, G. (1957). *Vet. Rec.*, **69**, 236.
OLSEN, O., WILFORD and BRACKEN, F. K. (1959). *J.A.V.M.A.*, **134**, 330.
OLSSON, S. E. (1952). *Nord Veterinary Medicine*, **4**, 983.
——. (1957). *Cornell Vet.*, **47**, 194.
THORPE, E. (1957). *Vet. Rec.*, **69**, 555.
URQUHART, G. M., JARRETT, W. F. H., and O'SULLIVAN, J. G. (1954). *Vet. Rec.*, **66**, 143.

THE SPEAKERS' INTRODUCTION

The Chairman, Mr. P. R. Blair-Fish, introduced the authors, Mr. Lauder and Mr. Lawson, and called upon them to present their paper.

Mr. I. M. Lauder, in presenting the paper, said that when he and his co-author were asked to deal with thoracic diseases, they had chosen those which could be accurately diagnosed during life. In considering the presentation, it had been obvious that some belonged to the same group, e.g., those associated with oesophageal obstruction, with the major sign of difficulty in swallowing.

The possibility had then been seen of classifying the other conditions under an appropriate clinical sign, and when this had been done it had appeared to them to have the advantage of being related to the form in which most veterinarians were presented with the problem of diagnosis of an individual case—they were given a dog with a cough, a gross dyspnoea respiration, and from that point considered differential diagnosis.

As the authors had chosen to concern themselves with differential diagnosis, they had mentioned further conditions in which specific diagnosis was difficult or impossible to carry out, e.g., kennel cough. They had concerned themselves mainly with diagnosis, and no attempt had been made to describe any condition fully.

However, he wished to make special mention of *Filaroides osleri* infestation and tuberculosis of the dog. The former had a very well defined syndrome and might be quite widespread. Tuberculosis was associated so often with active human infection in the household that he believed the veterinary profession should notify their medical colleagues every time a case was confirmed. Tuberculosis in man in this country was down, but it was by no means out, and he thought it was a good idea to kick it when it was down.

The authors had made their own definition of "hydrothorax" and meant it to include inflammatory as well as non-inflammatory fluid.

The cause of feline influenza should be given as "one or more viruses". Dr. A. W. Gledhill, at the 1952 B.V.A.

Congress, had described the isolation of a virus from cats with a syndrome similar to that of feline influenza, that virus differed from the one isolated by Baker which was generally accepted as being responsible for cat 'flu. Two clinical syndromes might be later differentiated, but in the meantime it appeared that 2 distinct viruses were associated with one clinical picture.

Some of the text of the paper consisted of his personal impressions and opinions, and was not necessarily the teaching of their Veterinary School.

They taught their students to be critical, and at question and comment time later in the morning members could take the opportunity of finding out if the authors themselves really welcomed criticism.

The first part of the paper dealt with methods of examination. In the past he had had the impression that a medical G.P. using percussion and auscultation could diagnose the extent and degree of pneumonia in his patient with extreme accuracy and say with great confidence that there would be a crisis two days from that moment in the evening. When he had started practising and listening to dogs' chests he had failed to get anywhere near that. Looking back, he thought that he had wasted a good deal of time on the *post-mortem* examination of dogs trying to trace the source in the lung of some very marked adventitious sound that he had heard during life, but it had been difficult to correlate *post-mortem* and adventitious sounds.

While clinical methods gave highly significant results, the medical profession put much more reliance on radiological examination for chest illness. He thought that they should do likewise.

Mr. D. Lawson, in his presentation of the paper, said that he wished to show a series of slides illustrating the interpretation that was applied to the X-ray pictures. The making of slides for projection from X-ray plates was technically very difficult, and it had been made even more difficult by the fact that some of the X-rays from which the slides had to be made were not of the very best quality, particularly some of the older X-ray plates made some time ago.

The section which was most difficult to show up was the actual pneumoniac changes, and this had only been done in a limited way.

In the interpretation of radiographs of the chest the pathological changes which occurred in the lung and related structure caused two types of alteration which were visible, one being an alteration in shape and size of the organs, or of structures within the thorax, and the other being alterations in contrast.

Mr. Lawson then showed the slides.

The Opener

Mr. F. G. Startup, who opened the discussion, congratulated the authors on an interesting and different approach to an every-day subject.

Diseases of the thorax, and their differential diagnosis, were a daily problem to those veterinary surgeons who were engaged in small animal practice, either as a specific disease of the chest, or as a symptom of some more generalised disorder. The differentiation of the various conditions could sometimes give rise to difficulty; and it was at times impossible on clinical evaluation alone. Consequently, they had to use other methods to obtain confirmation, and, of the various procedures available to them, radiology was becoming increasingly important.

He felt, however, that they must regard radiology as an ancillary procedure, and must not neglect other methods of diagnosis. History, examination and inspection were all-important, and an accurate diagnosis of many of the conditions described could be made without the resort to radiology. An estimation of the respiratory rate, percussion, auscultation and thoracentesis all helped towards a diagnosis, which was usually based not on one procedure alone, but on an evaluation of the total findings.

On the other hand, some of the conditions described could not be easily or definitely diagnosed without resort to radiology, and it was in those cases that it was of the greatest value. An additional diagnostic aid that had not been mentioned was bronchoscopy, which was sometimes of value in the diagnosis of Filaroides infestation and neoplasia.

Their knowledge of heart disease in the dog was very limited, and the specific diagnosis of a heart condition was the most important problem confronting the clinician in the management of these disorders. Much information could be gained by the methods already at their disposal, but it appeared that there had been considerable progress recently in the use of electrocardiography in the dog. He would be interested to hear from the authors whether they had any experience of this technique and whether they could give any opinion of its possible application and value in small animal medicine.

Because of the lack of subjective symptoms in their patients, they had to rely to a great extent on outside diagnostic aids to substantiate a clinical diagnosis. In the field of chest disease, the most valuable of those aids was undoubtedly radiology. Radiology could disclose those lesions that they could not detect on examination or which did not produce clinical symptoms; and could help in the differentiation of those conditions in which the clinical symptoms were identical.

Radiographic examination of the chest enabled them to study the lungs, diaphragm, thoracic oesophagus and the outline of the heart and great vessels. It was important, however, that they could interpret the normal before evaluating the abnormal. A certain amount of skill was required in interpretation, and he thought that it was important to acquire a uniformity of technique.

In the radiology of the chest, the film must be exposed rapidly enough to stop movement, and, with that in mind, fast films and intensifying screens must be used, enabling an exposure of $\frac{1}{4}$ second or less to be adequate. Both lateral and A-P views of the chest were useful, and, in the radiology of the lungs, the standing position was sometimes of value. In the recumbent position, the motion of the thorax could be minimised by the use of a towel drawn taut across the chest, and by firm pressure applied to the nostril to stop breathing at the moment of exposure.

In his experience, hydrothorax was the most commonly encountered condition that had been considered under the heading of dyspnoea, and, although the diagnosis did not usually present any problems, the differential diagnosis was sometimes difficult. Cardiac disease was undoubtedly responsible for most cases, and he thought that they should perhaps distinguish between pulmonary oedema and pleural effusion. He would be grateful if the authors could say something about that, as to his mind cardiac disorders would be associated with pulmonary oedema, whereas pleural effusion would more often be associated with infections and would give a different radiological picture.

When considering dyspnoea, he thought that they should also include the condition of emphysema. In this, radiological diagnosis would be difficult, unless there was a concurrent pneumothorax.

When presented with a case in which a cough was the primary symptom, it was important to consider conditions other than those that had been dealt with. Conditions such as pharyngitis, bronchitis, asthma, heart disease and foreign bodies were normally easily differentiated by normal clinical methods; but radiography was essential for substantiation of a clinical diagnosis of bronchiectasis. A technique for the introduction of a radiopaque medium into the main bronchus of each lobe had been described. This outlined the bronchial tree, and was a useful technique for the demonstration of bronchiectatis.

The subject of tuberculosis was important. Although, clinically, it would sometimes appear not to be a problem, he was sure that the incidence in the dog was considerably higher than it would seem. This presented an important public health problem, and the development of radiological techniques in the dog for the diagnosis of the condition was an important advance. The clinical diagnosis of tuberculosis was often difficult, and diagnostic aids available up to now had not been very reliable. The authors mentioned the very high levels of urine protein that might be found in cases of tuberculosis associated with amyloid change in the kidneys. He asked whether such high levels were peculiar to this condition, or whether they might be found in other renal disease as well.

The positive diagnosis of lung tumours also presented difficulties clinically and in those cases radiographic diagnosis was of great help. The authors mentioned only secondary tumours, but primary neoplasms also occurred

in the thorax of which bronchogenic carcinoma appeared to be the most commonly reported. The connection of thoracic neoplasia with Marie's disease, and also the connection of this latter disorder with tuberculosis, must be remembered.

Filaroides infestation of the trachea and bronchi might be suspected clinically from the history and type of cough; but bronchoscopy and radiology could confirm the diagnosis. This was an interesting condition, and one that he had only seen—or at least diagnosed—twice in the past 10 years. In both his cases, which had not been connected, the source of infection had never been established, nor had the many in-contact animals apparently been affected. It would be interesting to hear from members present whether any cases had been encountered, or whether anyone had any theory of the mode of transmission of this parasite.

Valvular heart disease formed, of course, an important clinical group, of which undoubtedly incompetence of the mitral valve was the most common type. Tricuspid incompetence, usually associated with some degree of mitral disease, was also seen from time to time; but it appeared that stenosis of those valves was very rare. He thought that a diagnosis of primary heart disease was sometimes difficult to establish. Many of the more obvious symptoms, such as dyspnoea, cough, ascites, oedema of the limbs, tiring and cyanosis were often due to other conditions and diagnosis must depend on careful and accurate auscultation.

To his mind, congenital cardiac disease in the dog was not important clinically, although the few cases that one did see from time to time were always of great interest. Many types of congenital abnormality had been reported, and they should always be suspected when a puppy was presented with evidence of heart involvement. It appeared from the literature that the most commonly occurring lesion was that of patent foramen ovale, which rarely gave rise to clinical symptoms and was of little importance. Clinically, the most common defect seemed to be a patent ductus arteriosus, diagnosis of which was based on the presence of a continuous, systolic and diastolic murmur, heard most clearly at the third left intercostal space—the co-called "machinery murmur"; and, in cases in which the ductus was widely patent, a high pulse pressure, producing the co-called "water-hammer pulse".

Other congenital defects that might occur included persistence of the right aortic arch, causing constriction of the oesophagus, as described by the authors, and pulmonary stenosis, which had also been described. Although, as it had been said, such a condition would produce gross murmurs and an enlarged heart, he thought that it would be difficult to arrive at a positive diagnosis by ordinary clinical means. The authors mentioned the use of angiocardiograms, and he would be grateful if a little could be said concerning the application and uses of that technique.

The diagnosis of an oesophageal foreign body would not usually present any difficulty. The history and the application of a meat test would usually be sufficient, and an X-ray examination, possibly with the use of barium, would enable the exact position of the obstruction to be determined. The removal of bony foreign bodies from the thoracic oesophagus by means of an oesophagoscope and forceps, he regarded as highly dangerous. The method of choice would obviously depend on the exact location of the bone, but many cases could be dealt with successfully by either a presternal oesophagotomy or via a paracostal gastrotomy incision if the foreign body was very close to the diaphragm. He thought that the operation of trans-thoracic oesophagotomy was rarely needed, but was the operation of choice when the bone was between the heart and the diaphragm.

The condition of mega-oesophagus would appear to be relatively common in the Alsatian, in which it was thought to be an inherited defect. Having encountered that condition, and been at a loss to know what to do about it, he would be grateful if the authors could give a brief outline of the technique in the cardioplastic operation devised for this defect.

In conclusion, he thanked the authors for their most interesting paper. When one was presented with a patient with thoracic symptoms, the diagnosis was not always easy, and he was sure that this paper would help them to reach an accurate conclusion.

The Replies

Mr. I. M. Lauder, in replying to Mr. Startup, said that he thought E.C.G. examination might become very useful. Quite a bit had been done in Glasgow but he personally had done little. With the very variable sizes and shapes of the different breeds of dogs, and movement of un-anaesthetised animals, interpretation of readings was very difficult. He did not know the actual figures of the incidence of hydrothorax of cardiac origin and of inflammatory origin, but in his experience the main single cause had been tuberculous effusion. With regard to the characteristics of the fluid in the pleural cavity, there should be a marked difference between effusion due to primary heart trouble and inflammatory exudate. Many biochemical and cellular examinations had been made on pleural effusion but the results were not often of diagnostic value. The high urine protein levels were not specific to T.B. infection but such levels were usually secondary to amyloidosis which in turn was usually secondary to tuberculosis.

Mr. D. Lawson in his reply said that he was in complete agreement with practically everything that the opener of the discussion had said on the technique of radiology. It had been decided not to include anything on it in the paper, which was primarily concerned with diagnosis. On the question of the possible confusion of pleural effusion and pulmonary oedema, from the radiographic point of view the two entities should be quite distinct.

The presence of fluid in the lung substance causes change in contrast of the lung field in the same way as pneumonic consolidation, but the changes are not so marked. By contrast, fluid free in the pleural cavity causes separation of the lobes of lung from the thoracic wall and from each other, if the amount of pleural effusion is only slight, or if the effusion is extensive the large amount of fluid present will largely mask all the other structural outlines because of its high radio-opacity; the fluid level may be seen if the lateral projection is taken with the dog standing.

With regard to bronchiectasis, they had not as yet detected the presence of this in dogs. This condition, which was relatively local in certain areas of Canada and the U.S.A., might be associated with certain infections there. In Glasgow they had done some work on bronchograms, but they did not think these had any very valid clinical application at the moment.

Bronchoscopy was certainly useful in visualising the lesions in *Filaroides osleri* infestation and the authors had used it extensively.

With regard to secondary and primary lung tumours, Dr. Cotchin had mentioned in his paper delivered the previous day, the fact that primary bronchial-carcinoma was relatively uncommon in the dog. There had been a small number of cases in Glasgow, and some of them had been diagnosable radiographically, but not all. They did on occasion develop large primary tumours in the lung which were then visible as single masses. The other feature shown up on a number of occasions, even when the primary was not clearly visible, was in the presence of secondary deposite in the bone. So far as the authors knew, these were the only secondary tumours in bone that they had had.

Angiocardiography was a highly specialised technique and unfortunately it required very expensive apparatus which was normally not available. The ones that had been done for them in Glasgow had been done in one of the human hospitals where they had special equipment for this type of work. Interpretation of angiocardiograms required the recognition of a number of factors.

The time taken for "dye" to pass through the right heart, lung field and left heart must be known and note made of retention of the "dye" in the various cardiac chambers.

The procedure employed in the operation of oesophagocardioplasty is to destroy the function of the cardiac sphincter by exposing it *via* trans-thoracic approach and opening of the hiatus oesophageus on one side, then incising through the sphincter longitudinally through the distal end of the oesophagus and the upper end of the stomach. The incision is stitched transversely so that the sphincter is fully relaxed.

Mr. Lauder said in reply to Dr. Cotchin that he accepted the criticism of the use of the term "hydrothorax". In practice one had a dog with fluid in its chest, and tried to find the cause. While doing so, it was convenient to apply some label to the case and "hydrothorax" was the one they used, although not with strict accuracy.

With regard to *Filaroides osleri* infestation, they had some cases on the go for 2 years. Four 7-year-old brood bitches from an infected kennel were destroyed without having shown any signs of illness and 3 had very small nodules at the bifurcation of the trachea. It seemed likely these nodules were caused by *Filaroides osleri*, but the histological report was not known.

Mr. Lawson said that Dr. Cotchin had reported some cases of hypertrophic pulmonary osteo-arthropathy associated with primary pulmonary tumour. So far this association has not been noted in Glasgow and the long term results of pulmonary lobectomy had not, as yet, been very encouraging.

In reply to Mr. Ingram, he was afraid that he could not claim any personal responsibility for the good radiographs. They were fortunate in the Veterinary School in Glasgow in having a radiologist who paid a lot of attention to getting good results. The techniques employed were almost always screen techniques using fast films and standard screens. One feature used fairly carefully was that the distance of exposure was a fairly long one, normally 36 inches. Many of these technical factors were of very great importance, but whether one could employ them or not depended primarily on the set up that one had. The employment of a Bucky grid, for instance, was not generally possible with a small portable machine, since it would make the exposure too long. The machine in Glasgow would go down to very short exposures, which helped very considerably, since it had enough power to use those very short exposures.

Mr. Lauder in reply to Mr. Wright said that he was sorry he had not the history of the Alsatian shown in the film at his finger tips. It had tuberculous pleurisy with effusion and it was rather common for an owner to wait until dyspnoea started before seeking veterinary attention. As the organism could affect any organ what the owner noticed of preliminary signs could vary a good deal. Before the advent of distressed breathing it was common to hear the dog had shown a little inappetance, dullness, loss of weight, and a cough during the previous two months.

The history of the miniature poodle with Filaroides which had been shown on the film was that it had been coughing for 6 months. It was in breeding kennels which had no direct contact or dog interchange with known infected kennels. A veterinary surgeon in general practice visiting the kennels for another reason had mentioned to him that one of the young dogs had been coughing for 6 months and recently the cough seemed worse though the dog was otherwise well. This history, in a breed he had previously seen infected, seemed unlikely to have been caused by anything other than tracheal worm infestation and he asked for X-ray confirmation, which was available.

The Chairman, in thanking the authors of the paper and the opener of the discussion, said that the paper was a very good example of the team work which they had come to associate with the Glasgow Veterinary School in producing original research work.

Plenary Session

Principles and Practice of Disease Control

BY

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IN Great Britain the first veterinary department was set up as a committee under the Privy Council in 1865 to deal with rinderpest, which had been brought to the country by cattle imported from Russia. At this time there was some legislation to deal with sheep scab and sheep pox and giving general authority to control the movement of animals, meat, hides, fodder, etc., in the interests of disease control. Two years later contagious bovine pleuro-pneumonia is first mentioned in legislation and in 1869 an Act dealt with rinderpest, pleuro-pneumonia, foot-and-mouth disease, sheep pox, sheep scab and glanders. Foot-and-mouth disease had existed in the country since 1839, pleuro-pneumonia since 1840, and anthrax, sheep scab, swine fever and rabies for an unknown period. Sheep pox was first introduced in 1847 and it was eradicated in 1850. There were subsequent introductions between 1862 and 1871, but the disease did not become established as each case was promptly dealt with by the "stamping-out" method. In 1883 an agricultural department, embracing the existing veterinary department, was set up under the Privy Council and six years later in 1889 the Board of Agriculture was formed; this became the Ministry of Agriculture and Fisheries in 1919. The veterinary department continued as the Diseases of Animals Branch of the Ministry of Agriculture until 1938, when the Animal Health Division was formed. It took over work which was already being done by local authority officers, notably on the veterinary public health side, as well as the duties concerned with the eradication of bovine tuberculosis in Scotland which were the responsibility of the Department of Agriculture for Scotland.

Following a report by a Royal Commission on Scottish affairs, the Scottish Department assumed responsibility for administration of certain animal health matters in Scotland in 1955, but the veterinary staff remained as a single unit under the direct control of the Chief Veterinary Officer, and the notifiable diseases other than bovine tuberculosis continued to be dealt with from the London headquarters.

Of the diseases which have been mentioned as occurring in the nineteenth century, sheep pox was eradicated in 1850, rinderpest in 1877, contagious bovine pleuro-pneumonia in 1898, rabies in 1922, glanders in 1928 and sheep scab in 1952. There remain as a problem to us in 1959 foot-and-mouth disease, anthrax and swine fever. Other diseases which have been eradicated are epizootic lymphangitis, which was introduced with horses returning to this country from South Africa in 1901, and eradicated in 1906; parasitic mange in horses was eliminated in 1948.

The stamping-out policy adopted in 1892 has always succeeded in eliminating foot-and-mouth disease, but the disease is reintroduced from time to time.

While the importance of some of these diseases of last century must not be over-emphasised, it should be realised that the losses from rinderpest far exceeded anything we have encountered this century. For example, some 90,000 animals (cattle, sheep, pigs and goats) were slaughtered during the foot-and-mouth disease outbreaks of 1951-52, whereas half a million of our cattle died of rinderpest in a year and a half after it was first introduced.

The disease situation in the nineteenth century is also of interest, because, with their comparatively limited knowledge of the causal agents of disease but with careful study of the apparent methods of spread and the application of a bold policy of stamping out infection, our predecessors were able to eliminate these diseases and pave the way for such work as we have been able to do in controlling and eliminating the less spectacular diseases.

The importance of the control of movement of animals, for example, was very fully appreciated. Where it is possible to prevent unnecessary movement and to authorise only essential movements of animals, there is ample evidence that spread of disease can be very largely controlled. Control of movement is one of the major contributions to a successful application of the stamping-out policy for foot-and-mouth disease. Indeed, it was the good effect on the incidence of foot-and-mouth disease resulting from movement controls imposed to deal with rinderpest that encouraged the authorities of the day to tackle foot-and-mouth disease. In more recent years it has been noted that in areas under movement control for foot-and-mouth disease the incidence of swine fever rapidly diminishes, and on those occasions when the application of area movement controls is justified in connection with swine fever itself, we have never failed to bring the incidence down quite rapidly. It is not only a question of preventing individual movements between farms, but much more particularly prohibiting the aggregation of stock, e.g., in markets or fairs, where by close contact infection is spread throughout a large number of animals which are in turn distributed over a wide area and may carry the infection with them.

Up to the time of the formation of the Animal Health Division many of the duties of the veterinary services in Great Britain were concerned with the public health. However, a small veterinary staff, to deal essentially with scheduled diseases and general measures of disease prevention, had functioned under the Veterinary Department of the Privy Council and subsequently under the Board of Agriculture and the Ministry of Agriculture. Veterinary officers were employed by local authorities. They carried out a number of duties under the Diseases of Animals Acts and had a large part in the elimination or control of many diseases. Glanders is a notable example. Their main functions, however, were to carry out routine examina-

tions of dairy herds with the primary intention of identifying cows clinically affected with tuberculosis and, in consequence, possibly contributing infection to the milk. In addition, local authorities were responsible for the tuberculin testing and supervision of milk production in herds from which they authorised the sale of milk under special designations. At the end of 1934 there were 980 T.T. herds [England 597, Wales 102, Scotland total 281 (117 certified)]. Many of the veterinary officers were also concerned with meat inspection.

I have thought it worth while to mention very briefly the achievements of our predecessors in eradicating and controlling disease, because they set us the example of eliminating infections wherever that is possible and because, had the major diseases which they eradicated been present in the country, we could not have begun to tackle the more insidious diseases.

What I propose to consider in rather more detail is the position in which we find ourselves in 1959 and the principles of disease control which we must accept if we are to make the contribution which the great advance in veterinary knowledge demands of us.

It is fully realised that a great deal of disease control in Great Britain in the future will continue to be done privately by veterinary surgeons in practice. By far the greatest value of the "Four Diseases Scheme" of the war years was to encourage the acceptance of the principle of the control of disease on a herd basis. This approach must continue and must be further developed so that all classes of farmers come to realise the benefits to be derived from the proper use of veterinary advice directed to keep the flocks and herds free from disease. However, in this address I am not concerning myself so particularly with the important work which is bound to be done in this way as I am with those diseases which, because of rapid spread, serious loss to agriculture or public health significance, come under some form of legislative control.

Veterinarians in any country are responsible primarily for the control and elimination of diseases of animals. They should also ensure the comfort and well-being of the livestock population whether it be farm animals, so essential in the agricultural economy, or animals maintained for man's pleasure. An important feature of the work must obviously be the prevention of the introduction of disease with imports, whether they be livestock, livestock products or materials which might be contaminated. Incidentally, but none the less important, there is the public health side of the work. In the narrower sense, veterinary public health work reduces the risk of infection spreading from animals to man and this is of real importance with a number of diseases; but it has a wider application by contributing, through good control of animal diseases, to the adequate supply of those high quality foods of animal origin which help to provide a high standard of living for a populace.

In the first place, it is clearly necessary that a sound organisation be set up to undertake this work. In this country the Animal Health Division of the Ministry of Agriculture, Fisheries and Food and the Department of Agriculture for Scotland carry the prime responsibility. A field organisation must be supported by research establishments and there must be teaching institutions to provide a sufficient, adequately-trained veterinary staff.

The organisation of the Animal Health Division is well enough known to all of you, but perhaps I may be allowed to mention what I consider to be the most important elements in it.

1. The fact that the veterinary staff is common to the whole of Great Britain ensures uniform disease control measures which may be systematically applied throughout the whole of the area over which livestock moves freely. It provides a better career prospect for the staff.

2. The provision of research facilities within the Division and the existence of the Veterinary Investigation Service, in addition to the field staff, provides for all aspects of disease control. It divides the work into manageable departments which, however, collaborate closely together and are all under the general control of the Chief Veterinary Officer.

3. The employment of veterinary surgeons in practice as part-time inspectors provides a veterinary staff which bears some relationship to the local stock populations and assists in maintaining a service of private veterinary surgeons in the more remote, less heavily populated, parts of the country.

4. The deployment of whole-time field staff in regions and in divisions (roughly counties) provides an adequate geographical distribution and the Regional Veterinary Officer is in a position to co-ordinate all the work of the veterinary staff in his region.

5. The good relationship between the administrative and the veterinary staff is an outstanding feature of this organisation. Each has an important specialised role, but their duties are complementary; the combination can only have its full effect when there is mutual respect and an appreciation of the differing problems.

6. The assistance given by local authority organisations, particularly the police, is indispensable. Police Constables, as such, accept notification of suspected disease. Inspectors under the Diseases of Animals Acts, who in most cases are members of the police force, have important duties in connection with general disease of animals legislation and with regulations dealing with notifiable diseases.

7. Other lay personnel, technical assistants, available for a number of wholly routine duties in the field, relieve the veterinary staff and allow their attention to be given to more strictly veterinary matters.

Besides a sound organisation, it is obviously essential that there is a basic knowledge of disease and that methods of control are well understood. Control methods used in the field may continually be reviewed and revised in the light of experience; and, so that this may be done, it is essential to study the field work as operational research and to co-ordinate the results at headquarters.

Over the years an efficient method of dealing with notifiable diseases has been evolved. However, amendments continue to be made. For example, in 1952 it

became obvious that the virus of foot-and-mouth disease had been conveyed to calves being fed as a routine on milk at Crewe Junction. A regulation requiring dried milk to be used for feeding calves in transit has been introduced. The Record of Movements Order which requires farmers to keep a record of the movement of animals on to and off farms, the Boiling of Animal Foodstuffs Orders and the Transit Orders (so far as they relate to disease prevention) are all examples of regulations made as a result of an obvious need disclosed in the course of the application of disease control measures in the field.

Extensive laboratory research and experimentation are essential and facilities are needed to inquire into practical problems which arise in the field and to which a quick answer is required. The disease control services must be nourished and sustained by research work of one kind or another.

The primary function, therefore, of the veterinary laboratories of the Ministry at Weybridge and Lasswade is to work mostly with those diseases for which there is legislative control and to provide a diagnostic service for them. A notable exception is foot-and-mouth disease. For obvious reasons this disease is studied at a separate laboratory and the Animal Virus Research Institute at Pirbright is responsible for all the work on this disease, including laboratory diagnosis. It is necessary for the Ministry laboratories to study diseases which are apparently of economic importance. While they take a full share in research into animal health problems, they can only undertake a very small proportion of the research work that is necessary. A great deal is done in other institutes. The tendency has been for this work to develop in research institutes either set up by the Agricultural Research Council or supported by its funds. With the inclusion of the veterinary schools in the universities from 1949 onwards more research has developed in the schools. Meantime, funds are provided as special grants for particular pieces of research over a short period by the A.R.C., or from funds obtained from a variety of sources through the energies of the senior staff of the university veterinary schools and to an extent from the limited funds available in universities for work in all faculties.

It may well be advisable in the future to provide means under which research could be regularly supported in the veterinary schools in such a way that it could be planned on a long-term basis allowing for organised training of research workers and so that it could reduce the burden of routine teaching and serve as something of an inspiration to the staffs.

This would surely allow the schools to exert a much greater influence in the broad field of veterinary activities. From such a background graduates could emerge fully aware of the problems facing the profession and with some judgment on the choice of the particular line they wish to follow.

If the results of research work are to have an impact on disease control, they must be made available not only for application by the official veterinary services but so that the veterinary surgeon in practice may take advantage of them in keeping himself up to date and applying the most modern methods of disease prevention or treatment. Only if this is well done can research play its full part and function as one of the important tools in disease control. The V.I. service has a considerable part to play in this connection. Congresses and conferences also assist: refresher courses at the schools and in laboratories might well be further developed.

I have already mentioned the control of imports to prevent the introduction of disease. The fact that the major diseases have been eliminated from this country makes it essential that we should endeavour to ensure that they will not be reintroduced. It is a matter of history that the only disease, mentioned as having been eliminated but which has been periodically reintroduced to the country from time to time, is foot-and-mouth disease. This occurs because the infection is capable of being carried in several ways besides in the living animal and in some ways which cannot possibly be prevented; as, for example, by birds. The import regulations have been successful in maintaining our freedom from the other diseases.

It is well known that dogs and cats are quarantined for a period of six months to protect us against the reintroduction of rabies. The six months' period is not too long. Air travel has reduced the period following possible contact with infection by the number of days occupied by a sea voyage. On two occasions disease has developed in dogs in quarantine after the six months' period had elapsed. We have frequently been criticised for maintaining the six months' quarantine period in view of the modern method of controlling rabies by vaccination. Vaccination, even when properly carried out, is not always effective and there is sometimes difficulty about certification. We have not, therefore, considered that there should be any alteration in the requirement of quarantine for a six months' period. Equally we do not consider that an exception should be made to exempt a particular animal from quarantine, even if at first sight it appears that a good case has been made for a concession. In order to protect the human and animal population in this country from the risk of this horrible disease, the quarantine of 1,900 dogs and 240 cats each year is a small price to pay. The consequence of the introduction of this disease does not justify any exceptions.

The importation of live poultry or hatching eggs and of poultry carcases is also prohibited from countries where such diseases as fowl pest are present. Since these orders were made there is no record of introduction of infection in spite of alleged irregularities in the import of hatching eggs.

It is only from Ireland, the Channel Isles, the Isle of Man and Canada that we accept regular imports of farm livestock without precautions other than inspection, a short period of detention at the landing place and detention for six days on the farm. From Commonwealth countries we accept pedigree livestock with suitable precautions which are laid down to meet each particular importation. Otherwise imports of farm animals are only made for exhibition and exceptional purposes. Import is only authorised if it has been shown that the animals are likely to be of benefit to a particular breed or to agriculture as a whole. The number of such importations is very limited. The risk of introducing disease is balanced against the potential benefit of the import. Imports would not be accepted

with even a remote risk of such diseases as rinderpest, contagious bovine pleuro-pneumonia or blue tongue; nor would imports be authorised from countries where animal disease control is considered to be so inadequate that the true disease position is unknown. Careful precautionary measures are laid down to meet the circumstances of each case. They may include certification of the health of the animals themselves, including negative test results for tuberculosis and brucellosis; freedom from disease on the farm and in the district of origin; and quarantine of the animals before shipment for a period long enough to cover the incubation period of a particular disease and so that any latent infection in one or more of the animals may become obvious when they are brought into close contact with those that are susceptible; quarantine on arrival in this country where healthy, susceptible home-bred stock may be maintained in close contact with the imports in order to act as indicators of the presence of any latent infection; a repetition of tests or additional tests during quarantine in this country. Importations which have been made in recent times include pedigree animals of polled breeds from the United States, Friesian cattle from Holland and Landrace pigs from Sweden.

The regulations contained in the Diseases of Animals Acts allow for a degree of flexibility of decision about imports of farm livestock. Except for imports from Ireland, etc., no hard and fast rules could be contained in a general order, since several species are involved and a variety of diseases must be considered, depending on the country of origin. Our system under which a special order is required specifying the precautions which are to be taken for each import has, therefore, much to commend it.

In recent years the precautions against introducing infectious diseases with imported livestock have apparently been successful, with the exception of the introduction of atrophic rhinitis with Swedish Landrace pigs. That a form of this disease was already present in this country has transpired in the course of inquiries and in surveys which have been conducted since that time. Other conditions, genetic in origin, have, however, been found in imported stock.

It is necessary for economic and political reasons to import animal products of one kind or another. About 40 per cent. of meat—beef, mutton and pig meat—has to be brought from abroad, and it is impossible to obtain this quantity from sources which are completely safe from the animal health point of view. Approximately half our primary outbreaks of foot-and-mouth disease are believed to have their origin in imported meat from South American. Again there is ample evidence that fowl pest was introduced with poultry carcases from the continent of Europe in 1947; about 20 per cent. of poultry meat is imported, but it now comes from sources reputed to be safe. At one time imports of American pig products were responsible for a high proportion of primary outbreaks of swine fever.

It is quite obvious that if decision about importation depended only upon animal health considerations much of the material of animal origin at present brought to this country would not be accepted. Patently, other factors have to be taken into account, and they include the overriding need to ensure that the populace is well fed (the empty-belly argument). Questions of trade agreements must obviously be looked at and certainly the quantity, quality and price of the product are important. For example, there is nowhere in the world from which we could obtain high quality carcases of beef in great quantity at such a reasonable price as we do from Argentina.

With this background every effort must be taken to reduce the risks of introduction of disease through importation of animal products. Broadly, a licence is needed to permit the landing in Great Britain of a carcase or part of a carcase of swine, cattle, sheep, goat or other ruminating animal. To reduce licensing to a minimum and to interfere as little as possible with trade, the Order contains a series of schedules which set out the products or countries which are exempted either unconditionally or conditional upon certain safeguards which are specified. Alterations in the schedule may be made from time to time and are based on a knowledge of the altered disease position in the particular country. Special arrangements under the Bledisloe Agreement are made with certain South American countries.

This agreement was entered into between Great Britain on the one hand and Argentina, Uruguay and Brazil on the other. It provides for certification of health of animals on the farm of origin, inspection while in transit and in markets and again on arrival at the frigorificos. It covers disinfection of the transport vehicles. Its most important provision is probably that it lays down that if any animal in a troop is affected with foot-and-mouth disease, no carcase of any animal in that troop may be exported to Great Britain. Two senior members of the veterinary staff are stationed in Buenos Aires.

It should be noted, too, in connection with swill that it is not only imported material which may be dangerous. In 1952 it appeared that material derived from salvaged carcases may have been included in garbage brought into contact with animals, thus giving rise to a large number of outbreaks of foot-and-mouth disease. It was because of this that salvage of the carcases of apparently healthy stock which had been in contact with infection was abandoned. Swill may also be responsible for spread of swine fever from salvaged carcases and for spread of fowl pest from carcases of birds slaughtered before disease was reported or discovered. In the U.S.A., boiling of garbage made an essential contribution to the control and apparent elimination of the pig disease, vesicular exanthema.

Anthrax infection may be imported in feedingstuffs or fertilisers. Bone and meat meal is often associated with outbreaks but other substances, including oil cakes, may be implicated. After the marked rise in incidence in 1956, when 1,245 outbreaks were confirmed, some firms have been using whale meat as a source of protein. The number of outbreaks has been much lower in the last two and a half years. The disease does not spread readily in this country, as illustrated by the fact that the number of animals affected per outbreak is only a fraction over one. Careful disposal of carcases and of contaminated material with careful disinfection of the part of the

buildings concerned has prevented the establishment of infection in Great Britain, where the climate is temperate. There are a few small areas that receive the overflow of rivers into which effluents from tanneries discharge, where grazing stock may regularly succumb to anthrax unless protected. A spore vaccine which is very effective is available for the purpose.

The comparatively minor incidence of this disease does not justify any general vaccination programme, nor does it justify undue interference with the import of feedingstuffs and fertilisers, since stoppage of the imports would cause more economic loss to agriculture than that resulting from the existing incidence of anthrax.

A Committee of the Economic Advisory Council reported on cattle diseases in 1934; and the diseases which were then considered to be of the greatest importance were tuberculosis, contagious abortion, mastitis and Johne's disease. The Report also mentioned the serious losses arising from infertility. As you all know, a great deal of work has been done subsequently on all the diseases mentioned. We are within sight of the eradication of bovine tuberculosis; contagious abortion has been brought under a measure of control by vaccination, encouraged by a voluntary scheme; mastitis, while perhaps even today one of the most important diseases of cattle, is much better understood and one form of it at least, streptococcal mastitis, can be controlled by the use of modern methods of treatment on a herd basis; Johne's disease is still a very serious problem. While infertility may be a heavy burden on the dairy industry, progress has certainly been made in regard to the infections which can cause it. The other factors influencing the breeding ability of cows can be studied on a wide basis in herds using artificial insemination, because the level of fertility and the health of the bulls are sufficiently well known to be calculated.

It will have taken us twenty-five years to bring the whole of the country to the stage when tuberculosis has been eradicated in the sense that each area is an attested area—probably in October, 1960.

The bulk of the tuberculin testing has been done by part-time inspectors. Whole-time veterinary officers have shared in the testing and have been charged with carrying out the first official test in each herd before it became attested; they have had to approve the premises before herds were accepted and have been responsible for the inquiries which have followed reinfection in attested herds. Research workers have been engaged in perfecting the tuberculins which are used, and we are now in a position to claim that each successive batch of tuberculin is virtually the same as its predecessor. Administrators have been concerned with the legislation which has provided the authority under which all the work has been done; and farming interests have gradually become so co-operative and so anxious to encourage eradication that for some years it has hardly been necessary to publicise the Attested Herds Scheme as was so necessary in the first few years. As the voluntary scheme developed and the stage was reached when some 20 per cent. of all the cattle were in attested herds, it became possible to introduce the Area Eradication Plan. The aim of this plan was to set up large areas in which the tuberculin test status of all the herds was equivalent to that of attested herds. In such an area it was expected that the reinfection rate would be reduced, and so it could be possible to test herds less frequently and to allow free movement of animals between herds, except from those in which reactors had recently been found; this would eliminate the need to provide Movement Permits for inter-herd traffic in livestock—formerly an enormous task.

When an area becomes attested it is protected against the invasion of infection in exactly the same way as is followed for an individual attested herd. In practice, it has been found that the weight of infection in the herds in attested areas is considerably reduced compared with the incidence in attested herds outside such areas. It is only about a quarter of the earlier incidence in attested herds and about half the current incidence of reactors in attested herds. The area plan is apparently having the desired effect. Approximately 0·11 per cent. of cattle are classed as reactors in attested areas.

Here, then, is evidence of a scheme which has been successful in eliminating a troublesome disease widespread throughout the country and well entrenched, particularly in our dairy herds. In 1936 we slaughtered 23,716 animals clinically affected with tuberculosis or giving tuberculosis milk; the average each year from 1936 to 1957 was 12,310. This only succeeded in giving a modicum of protection to the milk supply, even when a large number of veterinary surgeons was engaged in routine examination of dairy cattle and all the affected cows found at their inspections were slaughtered, in addition to those reported by owners and identified as a result of applying the biological test to the bulk milk of dairy herds. With all this effort the public health was not adequately safeguarded. Indeed, pasteurisation probably did more to prevent infection of children from cows' milk, but it could not be applied in rural areas. It is the approach to bovine tuberculosis as a disease of animals imposing a burden on agriculture that has succeeded in virtually eradicating the disease and consequently and incidentally in answering the public health problem. It is becoming evident that, when the whole of the country is one large attested area, we shall have to slaughter between 3,000 and 10,000 animals annually to maintain our herds for all intents and purposes free of tuberculosis.

While the main reservoir of infection in cattle will have been removed, at least for some years there is likely to be an element of reinfection arising from those animals with quiescent tuberculosis which had not sensitised them to tuberculin, but in which the lesion later breaks down and becomes infective. To guard against this type of case, fairly frequent herd tests will be required in the years immediately following the establishment of attested areas. There does not seem to be any serious risk of reinfection from other domestic animals, since the incidence of disease among them usually falls in parallel with the elimination of disease from cattle. There still remains, however, and there will remain for a number of years, the possibility that bovine-type infection acquired by workers associated with cattle may in turn reinfect the animals. This sequence of events has not been

encountered frequently, but it may continue as a minor hazard for a number of years in view of the fact that the life span of man is long enough to bridge the period between heavy infection and comparative freedom from tuberculosis in cattle. The danger, of course, becomes greater as the interval between herd tests increases, because of the opportunity for spread of infection which arises during the longer interval. The interval between tests must only be increased beyond three years if the experience of reinfection in our herds justifies it. We will be faced with some 3 million tuberculin tests per annum for a number of years after 1960. This is closely comparable to the number of tuberculin tests of all kinds, official and otherwise, carried out around 1950, when there were about 2,000,000 cattle in attested herds. Whatever the interval between herd tests, it will be necessary to ensure that each herd is tested at the agreed interval, that short interval tests are made on herds where reactors are found, that the testing history is ascertained and, if necessary, an immediate test is made in any herd to which infection is traced by the discovery of infection in milk, at an abattoir or a knackery or in an animal reacting in a herd to which it has been moved. The main reason for identification of all our cattle by tattoo mark or ear-tag is to facilitate tracing of the movements of individual animals, as necessary in following up the discovery of infection in many instances.

The main reason for success is the fact that we started with a scheme evolved to meet the known epidemiological facts about bovine tuberculosis. It has not been necessary to alter the rules originally made to protect tubercle-free herds, but the scheme has been altered to meet changed conditions as the work developed.

This scheme has been successful in dealing with bovine tuberculosis. We have endeavoured to make the test specific for this infection and have eliminated from herds only those animals which appear to be infected with bovine tuberculosis. This ignores those animals sensitised to tuberculin by skin T.B. and Avian type T.B., which do not as a rule pass from one bovine to another. It does not eliminate those that may be sensitised by *M. johnei* which is an entirely different problem.

Similar measures are not necessarily applicable to other diseases; for example, to contagious abortion, Johne's disease, which are two of the other diseases mentioned by the Committee in 1934. Objective tests may be developed for contagious abortion which are sufficiently specific and easy of application to warrant a scheme of eradication of infection. Efforts are now being made to learn enough about tests, particularly those which might be applied to the bulk milk of a herd to identify the infected herds before tests on individual animals are practised. With Johne's disease, on the other hand, there is no evidence of the emergence of a test which could be applied to the elimination of infection from herds. It appears that while some 17 per cent. of animals may be infected, only a minor percentage—surveys suggest 1 per cent.—succumb to the disease. With contagious abortion it would appear that a period of extended calf vaccination may be necessary before more specific measures are adopted and with Johne's disease it may be that vaccination will eventually have to answer the problem.

If legislative measures to control or eradicate disease are contemplated, a number of factors must be taken into consideration. Ideally, there must be a satisfactory method of diagnosis which does not depend only upon clinical signs or post-mortem lesions, and a knowledge of pathogenesis. There must be a promising method of ensuring elimination of infection on the premises involved. This may range from slaughter of the affected animals and observation of the contacts throughout a time longer than the incubation period to simple quarantine of the farm. There must be a means of knowing when it is safe to remove restrictions imposed upon the movement of stock from the farm and generally to prevent spread of infection. The precautionary measures may be costly and will obviously lead to a certain amount of interference with normal farming practice. The costs must be measured against the harm which is done by the particular disease. It must not be forgotten that direct loss from the disease itself is not the only item to be considered. There is the indirect effect its presence may have, for example, on the export trade in livestock; with scrapie, the impact on exports of sheep is far more important than the disclosed losses from the disease. Other things being equal, this would tend to favour the application of control measures. If there is a public health problem this will also tend to support attempts at control or elimination. If a disease has very recently been introduced to the country it may be that an early application of control measures might succeed even if similar measures could not be applied to the same disease if long-established in the country; in the former circumstances a valiant attempt would certainly be justified.

If establishment of herds free of a particular infectious disease is being considered the main essentials are that an objective test of some kind is available to ensure that infection is indeed absent from the herd and to decide which animals may safely be added to it. Sufficient must be known about the methods of infection to decide on the rules that must be applied to protect the clean herd.

Surveys may be carried out to assess the incidence and effect of a particular disease in order to decide whether the application of controls is justified. At this present moment we have nearly completed the second year of a disease survey of dairy cattle representative of the whole of Great Britain. The survey does not pretend to reflect the extent of infection in herds, but it does give an indication of mortality and morbidity and, as such, cannot but be helpful in guiding us towards those conditions which deserve the attention of the veterinary services. Its repetition at intervals will show trends in the disease position. It is our intention as time goes on to apply the machinery which has been set up to carry out these surveys to other species and to individual diseases.

Several vaccines have given excellent results in controlling a variety of animal diseases. They have been used against virus diseases and bacterial diseases with varying success. In some instances the effect of vaccination may be so dramatic that losses from a disease

which manifests itself in an acute form are reduced to an extent which demands changes in animal husbandry in a particular locality. Modern vaccines against rinderpest give the best example of this phenomenon, for the effect of the vaccine will so change the disease position in a relatively short period that progress in other branches of agriculture cannot keep up with it, and the result may be that insufficient foodstuffs are produced to maintain the increasing stock population. In such circumstances there must be a marked improvement in agricultural methods, and it may be helpful to develop a trade in meat and meat products in order to market the surplus stock. Nothing so dramatic is likely to happen in this country, but we are liable to be looked upon as a potential customer for surplus animal products from such countries and these products may carry a risk of introducing infection.

Vaccines have been produced which have very markedly reduced the losses from braxy, louping-ill and lamb dysentery. Other effective vaccines, like those against enterotoxaemia, are limited in their value by the difficulty of timing their use and, indeed, there is some evidence that they may be used unnecessarily under certain circumstances.

Rabies vaccination has also had a great impact in the prevention of this scourge among dogs in countries where the disease is endemic. Its value is, of course, limited, to the extent that it can only be used upon domestic animals, particularly dogs, and cannot have any effect on the situation amongst wild carnivores in which the virus can be maintained. For obvious reasons the use of rabies vaccine for animals is not authorised in Great Britain.

We have consistently avoided preventive vaccination against foot-and-mouth disease in our own stocks. This was fully supported by the Interdepartmental Committee which inquired into the situation and reported in 1954. It is, however, a matter of principle that we entirely support the use of vaccine as much as possible in countries where the disease is endemic or where the risks of reinfection are so great and so frequent that vaccination is the only possible policy, and we have derived indirect benefit from the employment of vaccines against foot-and-mouth disease in countries from which we were prone to become infected with the virus, notably as a result of the successful programmes in Holland, Denmark and Belgium. The research work which is done at Pirbright on the development of vaccines is of far greater value in this context than it is as a means of developing a method of disease control if the stamping-out policy should at any stage be hard pressed to eliminate infection from this country.

Vaccination against foot-and-mouth disease is well recognised in international circles as being but a stage in the elimination of the disease, which is unlikely of itself to bring about complete eradication. The European Commission for the Control of Foot-and-Mouth Disease in its overall plan, which was published in 1957, well recognises this fact and emphasises the need for measures other than vaccination and even for more specific methods to be used in substitution at a later stage. The plan recommends that:

"In those countries where a heavy weight of infection is present or where there is a continued threat of infection, the policy should be systematic vaccination. The system may vary in accordance with the circumstances in the particular country. It may be possible, for example, to adopt annual vaccination of all cattle stock in the country, or vaccination may be done around foci of infection or in threatened areas. When the number of outbreaks has been sufficiently reduced, vaccination may be supplemented by slaughter of animals on infected premises. Later, there may be a reduction in routine vaccination and the stamping-out policy may be adopted as the main method of control. This stage may not be reached in a particular country until its neighbours and countries from which imports, particularly of livestock, are made have reached a similar position. Only after this stage will it be possible to rely entirely on a stamping-out policy.

"It is obvious that sufficient supplies of trustworthy vaccines must be available and that there is a staff capable of organising and carrying out the vaccination. It must be emphasised that, whatever the policy, it is essential to control the movement of stock, to dispose of infected material and to carry out effective disinfection.

"The Committee was impressed with the success of the vaccination policy in those countries where it has been systematically applied. Not only has the number of outbreaks been reduced, but the weight of infection has been minimised, because, as a general rule, only young cattle and pigs have succumbed to infection.

"In order to have a stamping-out policy, the following points must receive careful application:

1. Early notification of a suspicion of the presence of the disease must be available.

2. A very meticulous system of dealing with such reports must be in force and must be readily put into effect—the system must include rapid diagnosis —immediate control of movement of stock over an area—immediate quarantine of the suspected or infected farm—tracing of all contact animals and slaughter of direct contacts on other farms, e.g., animals recently moved off the infected place and animals handled by the owner of the infected place or his veterinary surgeon after contact with infection.

3. Rapid means of slaughter and disposal of carcases on the infected place.

4. An organisation capable of dealing with all the detailed tracing of stock which is necessary.

5. A system of recording movements of stock must be permanently in force and enforced to allow of easy tracing of animals.

6. Visits to examine stock in the vicinity of outbreaks must be made to discover secondary outbreaks as early as possible and also to discover whether the first reported case is in fact the primary one.

7. There must be considerable elasticity in financial arrangements to allow of vigorous pursuit of the policy.

8. The farming community must be willing to co-operate in every aspect of the work.

"In most European countries a stamping-out policy, not supplemented by vaccination, is impracticable, but the Committee regards a stamping-out policy as the ultimate objective."

While of great importance in the control of animal diseases, vaccines are apparently unlikely to be effective in completely eliminating a disease. This does not, however, mean that the use of vaccines is to be discouraged. It means that the limitations of the use of the vaccines must be fully understood and appreciated not only by the veterinary profession but also by the farming public. There must be no false impression that disease will disappear automatically if vaccination is carried out. In this country we have included the use of vaccine in two voluntary disease control schemes—the Calfhood Vaccination Scheme against Contagious Abortion and the Swine Fever Registered Vaccinated Herds Scheme. In the former case the value of the vaccine flows from its ability to reduce the incidence of abortions, thus preventing losses of calves and to an extent avoiding the development of sterility in the cow. It becomes evident that the vaccine will not necessarily prevent the establishment of abortus infection in the vaccinated animals and that this infections may be in the udder and may be transferred from cow to cow at the time of milking. It must be made perfectly clear that the vaccine itself does not lead to the elimination of vaccinal or other strains through the milk. The importance of this vaccine, therefore, is to remove the worst effects of the infection and to precede eradication measures aimed not at reducing abortions but at eliminating infection. Before such measures are adopted, adult vaccination will have to be discouraged to avoid setting up permanent agglutinins in the blood of animals vaccinated as adults.

The swine fever vaccine was intended mainly for breeding herds to protect adult stocks against the disease and to provide from these herds a supply of vaccinated young stock which would reach the herds in which they are to be reared and fattened in a condition which would allow them to resist infection. It may be used also in herds of rearing pigs; but if it is so used the risks and the limitations must be fully understood by the veterinary surgeon and the farmer.

Only healthy pigs should be vaccinated. Immunity takes fourteen days to develop, and, consequently, the vaccine should not be used for pigs in contact with infection and pigs should be kept away from infection for fourteen days after vaccination. It will be appreciated that there may be exposure to infection in a market prior to purchase, in transport vehicles, when fed uncooked swill, during movement for breeding purposes, or at the time of castration. Satisfactory immunity may not develop in piglets vaccinated before they are three weeks of age, or in pigs which are vaccinated while they are harbouring some other infection or suffering from anaemia or nutritional deficiency. If any of these broad precautions are not taken, possibly at the insistence of the owner, the risks involved must be fully explained. Faulty vaccination technique and improper storage or dosage of vaccine may impair resulting immunity.

With the limitations in the use of vaccines, it is evident that disease control schemes based upon their use are less likely to be generally acceptable to farmers than a scheme like the Attested Herds Scheme. Vaccines are never 100 per cent. effective and generally it is the failures which tend to become publicised. Modern therapeutics provide valuable aids in disease control.

Just as one must realise the limitations of vaccines in the control and elimination of disease, so the extent to which modern methods of treatment may be exploited for the same purpose must be appreciated. The tendency for antibiotics to be employed other than as treatment in therapeutic doses for specific diseases has been deprecated by the veterinary profession. Recently evidence has been accumulating that unless therapeutic doses of these drugs are employed there is a very real danger of resistant strains of certain organisms emerging. It would seem to be a principle that should be well exploited, at least in the veterinary profession, that these useful drugs should not be abused in this way and that if an antibiotic is to be used as a growth promoter it should be a specified one with a narrow spectrum, so that any resistant organisms may remain susceptible to the effects of the wider spectrum drugs.

We have reached a stage in the control of animal diseases in this country where it is quite patent that in some instances much more research work is necessary before any means of prevention, control or elimination can be adopted. In other instances, it is necessary that the results of research work should be more widely publicised, so that the results may be translated into action by way of disease prevention and control on the farm. It may be that enough is known and the disease is of sufficient importance to justify the development of an official scheme or even of legislative control measures.

Less than enough is done to deal with diseases among pigs and poultry. There is, however, the prospect that after we have completed the major task of eradicating bovine tuberculosis it may be possible in various ways to encourage the profession to give more of its time to these species, in which there is obviously great scope.

Biochemical Aspects of Diseases of Livestock with Particular Reference to Enzyme Systems

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Summary. *Elements formerly believed just to be toxic hazards to farm animals continue to qualify as trace elements essential for animal life. A few years ago molybdenum qualified, and still more recently selenium. Molybdenum has turned out to be the "xanthine oxidase factor" needed by animals, and selenium has proved to be Factor 3 which is a partial substitute for vitamin E. In ruminants molybdenum shows interesting interactions with copper and with sulphate and we have found that it is antagonized in the lactating goat by tungstate. It is a constituent of xanthine oxidase which also contains iron and vitamin B_2 (riboflavin). Recent findings are described, including work of the speaker and his colleagues on the variations with species of xanthine oxidase in milk. The key position of the ruminants, both cow and sheep, in the food chain supplying man with Co as vitamin B_{12} are discussed, and reasons advanced for the ruminant's (cow's and sheep's) large requirements of Co compared with the small amount in the vitamin B_{12}, sufficing for man. Enzymatic functions of B_{12} are described. The effects of parasitism and of thyroxine in increasing B_{12} requirements are also discussed. Recent discoveries of the function of selenium as a substitute for vitamin E in the rat, chicken and pig are discussed, and reference is also made to other metals implicated in the etiology of diseases of livestock.*

THE number of enzyme systems in which trace elements are known to be involved has increased of recent years and quite recently molybdenum, selenium and zinc have become recognized as being of economic importance in animal production, selenium being the latest admission to the ranks of essential trace elements. I shall try to show where possible the connexion between animal disease and enzyme systems of which the trace elements cobalt, molybdenum, selenium and zinc form esential parts. The other trace elements, iron and copper and iodine, will be mentioned where they also participate in enzyme systems involving the first mentioned 4 elements whose names therefore head the sections of my discourse.

The Comparative Metabolism of Cobalt

The human ailment, pernicious anaemia, has been known as a clinical entity since 1849 and pining or vinkuish or vankuish in sheep in Scotland has been known as a clinical entity since 1807, yet it was not till 1935 that pining in sheep was shown to be a deficiency of cobalt and not till 1948 that cobalt was found in the extrinsic factor, lack of which Castle had posited as part of the cause of pernicious anaemia in humans. This extrinsic factor was isolated almost simultaneously by workers in England and U.S.A. The concentrates were pink in colour and tests showed that this was due to the cobalt which the antipernicious-anaemia factor (now usually called either vitamin B_{12} or cyanocobalamin) contained. Meanwhile other workers in the field of animal production had been searching for an animal-protein factor in certain fermented foods and in animal foods without which pigs and poultry and the experimental rat would not grow at maximal rate or with maximal efficiency of utilization of their food. Vitamin B_{12} is part if not all of this animal-protein factor. Ruminants did not seem to need this animal-protein factor. Indeed it was present in their dung which could cause stimulation of the growth of chickens which ate it. Vitamin B_{12} is a complex of cobalt and porphyrin. the cobalt exhibiting its hexavalent state with 4 links one to each N atom of the tetrapyrrole nucleus, one link to a cyanogen group and the remaining link to one of the nitrogens of dimethyl-benzimidazole. This benzimidazole is part of a nucleotide-like structure for its other nitrogen is linked, through an α-ribo-furanosido-group to orthophosphoric acid which is linked in turn through the oxygen of an isopropyl alcohol group to a propionamide sidechain of the tetrapyrrole on what is known as ring D. Many of physiological properties of the vitamin can be related to its structure and the chemical structure itself has been and still is a fruitful starting point for hypotheses as to its possible functions in the bodies of man and his domestic animals. Cyanocobalamin is a vitamin which contains a trace element as an essential constituent, and moreover contains a labile cyanide group. The cobalt in B_{12} is not labile and even after months of contact *in vitro* cobalt in B_{12} does not exchange with inorganic radioactive cobalt. The organic chemists have made some changes in the molecule. The CN can be replaced and so can the two methyls on the benzimidazole without loss of potency when the substance is injected. This is a marked contrast with the biochemistry of riboflavin which is inactive when its methyl groups are removed.

Oecologically B_{12} is the most interesting of the vitamins for it has shown how the highest form of life so far produced i.e. Man, is dependent upon the lowest form capable of a non-parasitic existence i.e. the bacterium; for B_{12} seems to be mainly a bacterial product. Thus Ericsson after having found B_{12} in seaweed, studied this discovery further and came to the conclusion that the B_{12} was really produced by bacterial epiphytes from which it was absorbed into the tissues of the seaweed. B_{12} has been reported to occur in small amounts in lucerne leaves, but probably this B_{12} is elaborated by the N-fixing *Rhizobium radicicola* which causes the nodulation of the lucerne

roots. *Rhizobium* has been shown when cultured *in vitro* away from its host to be able to synthesize B_{12}. In human food the milk of ruminants is a very important source of B_{12}. Here again, however, the source is not the ruminant's own tissues but the symbiotic bacteria which live in its rumen. In nature the ruminant must have been responsible for a peculiar food chain for such animals as pigs and chickens will eat undigested grains and seeds in the dung of cows and so incidentally supply themselves and, through their flesh, man with a ready-made source of the whole B-complex including B_{12} in which ruminant faeces are particularly rich. Through a very potent cource of B_{12} and other B-complex vitamins, ruminant faeces are of no use in this respect to their hosts; for B_{12} in all species studied in detail i.e. man, the rat and the pig, seems to have to be absorbed by the small intestine after combination with Castle's intrinsic factor. Thus synthesis in the ruminant caecum and colon would seem to be of no use to the host animal. The rabbit has by-passed this difficulty by producing from its caecum at night, faeces which it eats as fast as they leave the anus—and this coprohagy is the reason why it has not proved possible to produce a B-complex or B_{12}-deficiency in this species. An interesting confirmatory experiment in this regard is the recent observation that ^{60}Co when injected into the sheep cannot cure pinning in that animal but does nevertheless find its way into the caecum and colon where the symbiotic bacteria change it into cobalamins among which is vitamin B_{12} itself which escapes in the faeces.

Among micro-organisms some need B_{12} for their growth in certain man-made media while others do not. Indeed the needs of a bacterium (*Lactobacillus lactis*, Dorner) for B_{12} made the isolation of a potent concentrate from liver a simpler process in the U.S.A. (Rickes, Brink, Koniuszy, Wood & Folkers, 1948) than in England where Lester Smith (1952) and his colleagues had to rely entirely on clinical tests of human cases of pernicious anaemia until they suspected that the pink colour was indeed a property of the vitamin itself. Any exacting organisms may be used as the basis for microbiological test. Among them are *Lactobacillus lactis* Dorner, *Lactobacillus leichmanii*, a *Bacillus coli* mutant, the phytoflagellate *Euglena gracilis* and the brown flagellate *Ochromonas malhamensis*. This last organism is a protozoan and has become the one of choice because it responds in a similar way to man, pig and rat, to various cobalamins, some of which though active for the lacto-bacilli, the colibacillus and *Euglena* have no power to cause remission of relapses in sufferers from pernicious anaemia. It is among the bacteria that the chief industrial producers of B_{12} for human and animal use are found. Thus *Streptomyces griseus* produces not only the antibiotic streptomycin but also B_{12} thus obviating the use of ruminant liver as a source. Researches have shown that intrinsic factor is produced not only by the human stomach but by the stomach of the rat and pig as well. I do not know whether other animals' stomachs have been studied to see if they also produce intrinsic factors. There is evidence that though pig and rat intrinsic factors, like the factor from the human stomach, are active in facilitating the absorption of cobalamin by human cases of pernicious anaemia, the converse does not seem to be true and the human gastric factor seems not to aid the absorption of B_{12} by the small intestine of the pig or the rat—neither is pig intrinsic factor active in facilitating the absorption of cobalamin in the gastrectomized rat. Thus investigators of intrinsic factors have suggested that these factors may be species-specific. Intrinsic factors also have a high specificity towards cobalamin itself and, of those cobalamins which are effective by injection, only cyanocobalamin seems to have its intestinal uptake facilitated by intrinsic factors (Reisner, 1958). The chemistry of the intrinsic factors has not proved so simple as that of cobalamin itself, but concentrates of the pig factor have been prepared and it appears either to be, or to be combined with, a mucoprotein. Radioactive Co has been used to study the binding of vitamin B_{12} *in vitro* by intrinsic factors but such experiments are not as useful as they might have been because they have shown that the vitamin combines in non-dialysable, non-ultrafiltrable forms not only with intrinsic factor from the stomach but with a variety of proteinaceous materials. Thus intrinsic factor from human gastric juice binds B_{12} *in vitro* in non-ultrafiltrable form but so do human serum, human saliva, human colostrum and protein preparations from other organs. Thus this binding *in vitro* does not help in characterising concentrations of intrinsic factors but may perhaps help to explain how B_{12} assists protein synthesis in the liver.

The avid binding of B_{12} by living tissue and by proteinaceous material is the probable reason why conditions resembling pernicious anaemia have been observed in human carriers of the fish tapeworm *Diphyllobothrium latum* in whom vitamin B_{12} becomes permanently bound by the worm and thus is stolen from the host. (Nyberg, Grasberg, & Sippola, 1958; Nyberg, 1958).

It would appear that the course of vitamin B_{12} in humans is to combine with intrinsic factor on or shortly after leaving the stomach and then to be absorbed through the mucosa of the small intestine. Thence the B_{12} finds its way to the bone marrow which presumably in all animals is the chief site of its action in furthering erythropoiesis. The megaloblasts from which the red corpuscles arise do not seem able to mature properly in the absence of the vitamin. There is no way as yet of proving whether or not the intrinsic factor accompanies the B_{12} into the bone marrow in the normal course of events, but some investigators using cultures of bone marrow *in vitro* claim to have shown that B_{12} is more active in causing maturation of megaloblasts in such preparations when intrinsic factor is given with it. The greatest concentrations of B_{12} are found in the liver and kidney of most species. In prolonged deficiency in man the liver has poor reserves of B_{12} and the same is true of the hepatic Co reserves in sheep after prolonged deficiency of dietary cobalt.

The pig can be made deficient in vitamin B_{12} as also can the chicken and the rat. Deficiences are more acute and more quickly produced if young are raised

from parents which have suffered a partial deficiency of the vitamin. This point has been well demonstrated in the rat and the chicken. Indeed, in the chicken, partial deficiency causes hens to lay a higher percentage of eggs which will not hatch and the proportion of hatchable ones may be increased by injection of pure B_{12} into the eggs. In mammals (rats and man) there is good evidence for the transfer of B_{12} to the fœtus through the placenta.

Worked out on the basis of the known minimal requirement of vitamin B_{12} the human requirement of cobalt is minute, perhaps only 40 millimicrograms cobalt per day. By comparison the requirement of the sheep for cobalt is much bigger, perhaps 1 mg. per day, and perhaps it would be well to suggest some reasons why this might be so.

First is the fact that the sheep does not get vitamin B_{12} in its diet but depends upon its rumen bacteria for its supply. This means that the rumen bacteria get the cobalt first and process it to B_{12}-like compounds only one of which cyanocobalamin, is suitable for intestinal absorption. Thus, much Co will be lost to the sheep right at the start. The sheep manage to digest and absorb only a fraction of the incoming cobalt—the known richness of ruminant faeces in B_{12} is evidence of this. Probably the absorption of B_{12} is far from efficient in the presence of all the bacterial bodies which form such an important part of the sheep's diet. Whether the sheep's abomasum produces an intrinsic factor seems not to be known, but if it does, the mixture of bacterial, food and protozoal proteins with which it has to compete would on analogy with man, appear to militate against its efficient action. Another possible factor which will increase the sheep's requirements of cobalt is the fact that sheep habitually carry rather heavy burdens of blood-sucking worms in their alimentary tracts. These helminths, apart from necessitating more rapid blood formation to make up for the blood which they cause the host to lose may be specific storers of B_{12} which is therefore lost to the host. It is of interest that the fish tape-worm *Diphyllobothrium latum*, to which reference has already ben made, when infesting the human population in Finland can cause pernicious anaemia in them. If these tapeworms are killed by drying and are ground to a powder, the powder is found to be effective against pernicious anaemia and to be rich in B_{12}.

Recent experiments have shown (Stewart, 1951; 1953) that the main effect of cobalt shortage in the grazing sheep is a recession of the appetite. Being a disease of animals which exist entirely on grazing and which receive while on the deficient lands, very little or no supplementary feeding, pining in the sheep is a geologically determined disease (Dunlop, 1947; Underwood, 1956). Some soils contain too little cobalt for the grass and herbage on it to contain enough for the sheep's needs. The grass and plants themselves do not seem to need cobalt so that it is possible to have a good crop in a given season with even less cobalt in it than usual. In other soils the cobalt may simply not be in a form assimilable by the plants. This is the case in very calcareous soils such as those of Tiree off the Scottish coast, or on the coasts of Western and South Australia, where both cattle and sheep suffer from the deficiency. Dunlop showed the close connexion between geology and cobalt-poor areas in Scotland and Wilson and his colleagues have amplified his findings (1959), and Underwood (1956) discusses the same phenomena in Western Australia where for years sheep were not run at all near the west coast.

There can be little doubt that cobalt deficiency in the sheep is deficiency of vitamin B_{12} created by an insufficiency of an essential raw material, Co, for its manufacture by the rumen bacteria. Pining sheep can be cured by oral administration of cobalt or by injection of vitamin B_{12}. Injected cobalt has no effect unless it finds its way into the rumen. B_{12} by mouth in large doses can be effective but is of course quite uneconomic. Indeed the cure of pining in sheep in most parts of the world is no longer an academic problem. It is an economic one, for sheep whether in Europe or Australia are run on land where human habitation is sparse and where the number of musters of the animals for shearing and dosing for worms or dipping to kill ticks and other ectoparasites with their concomitant hazard of tick-borne diseases must be kept to a minimum. It would seem at first sight that cobalt could be given to the animals every time they are mustered. However cobalt given on such infrequent occasions would be useless. Like ascorbic acid in the guinea pig or man, cobalt has to be given little and often and the economic problem is how to accomplish this without undue cost of materials or labour. One dressing of the land with 20 oz. $CoSO_4$ per acre every 3 years is known to be preventive but this is not always easy to do on some of the very hilly land on which sheep graze. On large holdings in New Zealand dressing the pastures with cobaltized superphosphate from the air has met with some success but such a method is scarcely feasible in Scotland. Dewey, Lee & Marston (1958) in Australia have been using for some months now a new method of treatment which promises to be the answer to this problem. They have had made pellets called cobalt bullets. These are about 1 cm. wide cylinders 1 cm. long and are made of CoO_3 and clay. This has a specific gravity of about 4 and Marston and his colleagues have found that such a bullet tends to lodge in the reticulum, there to be very slowly dissolved and so to provide a continual trace of soluble cobalt for the animal's rumen bacteria which convert it to the form of vitamin B_{12} and so keep their host healthy. Of course even this method has its difficulties. An occasional lamb or sheep may regurgitate its pellet but it then starts to pine and is soon noticed from its poor condition by comparison with its fellows. In certain areas in Scotland pellets seemed to be less effective. Recovery of the pellets showed them to have become encrusted with a coating which prevented the CoO_3 from dissolving.

We have shown by physical, optical and chemical analyses (Owen *et al*, 1959) that the incrustations are, as indeed, Dewey *et al* suggested, made of the mineral brushite which has been reported before only in

guano (Dana & Ford, 1922) and in certain forms of human urolithiasis (Prien & Frondel, 1947). Since it is soluble in dilute acid perhaps incorporation of citric acid into the bullets would obviate this unexpected hazard. Experiments on these lines are now being prepared.

In certain bacteria, B_{12} has to be supplied in the medium only if sources of labile methyl groups are absent, e.g. when methionine is replaced by homocystine or homocysteine. Thus the power to catalyse transmethylation appears to be a property of B_{12}.

An even more important function of B_{12} seems to be to catalyse the production of methyl groups from formyl groups or from methanol in higher animals and this may be one reason for the part it plays in the anabolism of nucleic acids for it appears that in certain bacteria and even perhaps in man, uracil can be methylated to thymine (5-methyl-uracil) only when B_{12} is present. Moreover it has been known for a long time that such bacteria would grow without B_{12} if thymine or thymidine were in the medium and that these substances are also able to cause some remission of pernicious anaemia in relapse.

Wagle, Mehta & Johnson (1958, 1, 2) have recently showed that B_{12} may play an essential role in the synthesis by the rat's liver of proteins from the metabolic pool of amino-acids. Hoagland, Keller and Zamecnik (1956) had previously found a biochemical system in the rat's liver which synthesized protein when a mixture of amino-acids was supplied in the medium. For maximal activity the system needed to contain mitochondria from the liver and also an aqueous extract of what Hoagland *et al* (1956) call the "pH 5 enzyme system". Wagle *et al* (1958, 1, 2) have shown that protein synthesis in such systems is much reduced if the livers are from rats maintained on a diet poor in B_{12}, and they were able to restore the activity *in vitro* to normal by adding a mixture of amino acids and B_{12} to it. Without the B_{12} full activity was not obtained.

Wokes & Picard (1955) have suggested that another function of B_{12} has to do with the fact that although B_{12} occurs in the liver always as cyanocobalamin it can be supplied with equal effectiveness as nitrate, sulphate or hydroxo-cobalamin and doubtless in other salt forms which have not been tried. Hydroxo-cobalamin can form such compounds not only with sulphate and nitrate, but with nitrite, with H_2S and with thiocyanate. In all these compounds the CN group of the cyanocobalamin (i.e. of B_{12} itself) is replaced by the acid radicle mentioned. The presence of cyanide in the molecule of a metabolite essential for all animal species tested is something remarkable in itself and has led to investigations as to its function. Boxer and Rickards (1951) worked out a method of estimating B_{12} which was based on the release of cyanide from it. For this they had to devise an ultra micro method for estimating cyanide and were able to show incidentally that HCN is a normal constituent of the breath of both man and the rat and that its rate of production is proportional to the metabolic rate (1952). This immediately reminds one of the known antagonism of B_{12} and the chief hormone controlling the metabolic rate i.e. thyroxine, for it has been known for several years that in the presence of added thyroxine the rat's requirement of B_{12} is increased and this phenomenon has been used to increase the sensitivity of rat assays of vitamin B_{12}. Moreover B_{12} can increase the animal's resistance to hyperthyroidism. How cyanide arises endogenously other than from thiocyanate is an open question though of course particularly in herbivorous animals like the sheep there is a large intake in greenstuff and seeds but it does not seem to cause these herbivores much harm. Calves are still occasionally killed by cold mashes of fresh linseed meal because of the cyanide set free from the glucosides by maceration and lambs die in larger numbers than adult sheep when having access to poisonous herbages. Adult ruminants seem able to withstand far larger doses of cyanide than a non-ruminant could tolerate (see reviews by Owen, 1947, 1951, 1954, for references to the literature). Perhaps we have here yet another reason why the requirement of cobalt of the ruminant is so much larger than that of man. It might be thought that the chief site of detoxication of cyanide by the sheep was in the rumen but according to the work of Blakeley & Coop (1949) the liver is the chief detoxicative organ and this is where in most animals studied the chief store of B_{12} is found. It is clear however that the detoxication of cyanide in the sheep is carried out by some continously working mechanism for processing the incoming cyanide. In the rat Boxer & Rickards thought that, since B_{12} is a cyanide compound, and since it is equally active (by injection though not by mouth) whether it has or has not its cyanide radicle hydroxo-cobalamin should be the ideal antidote for cyanide. They therefore gave mice lethal doses of cyanide. When these were followed immediately by injection of hydroxo-cobalamin the mice survived. It has long been known that the enzyme rhodanese of Lang has as its function the transformation of cyanide (CN') into thiocyanate (CNS') in the presence of a suitable donor of sulphur such as thiosulphate (S_2O_3'') and it has been known for an even longer time that thiosulphate and thiocyanate are normal constituents of the body fluids of man and animals. Moreover unlike cyanide, thiocyanate and thiosulphate are comparatively non-toxic substances for CNS' is often used for the estimation of body water and for treatment of high blood pressure, and solutions of the thiosulphate used to be injected into pellagrins to relieve their symptoms in Sabry's method of treatment which was used by some physicians in the days before the discovery that pellagra was a deficiency disease. It was known that pellagrins were deficient in sulphur for their nail parings contained only half the normal amount of sulphur. Pellagrins also excrete more thiocyanate than do normals. It would appear that the body has a power to produce, probably in the liver, small amounts of cyanide, for cobalamins not having a CN group are transformed into cyanocobalamin after injection into the body. Evidently any excess cyanide produced endogenously or any cyanide coming from cyanogentic glucosides

in the food will be transformed into the relatively harmless thiocyanate by the liver. From time to time in the biochemical literature claims have been made that the body can produce an antithyroid substance, but none of these has been substantiated so far as I know. It is interesting therefore to speculate upon the implications of the known antagonism of B_{12} and thyroxine. For if Boxer and Rickards are right a stimulation of the metabolism by thyroxine should increase cyanogenesis and the resulting cyanide would stimulate the Lang rhodanese system to turn the excess of this into thiocyanate. Thiocyanate is known to be a specific antagonist of the elaboration of thyroxine in the thyroid gland but does not itself enter the gland. Hence this theory, of which Fig. 1 is a diagrammatic representation, would make the secretion of the thyroid gland self-limiting without the need to invoke the hypothesis of autogenesis of an anti-hormone. This theory of thyroid action has other consequences for it implies that the action of thyroxine is self-limiting only if the thyroid remains in circulatory contact with the rest of the body. It also implies that in the absence of a stimulus from increased metabolism CNS′ production will be minimal. It would therefore be expected that in a thyroidectomised animal the balance in the tissues between CNS and thyroxine would be very precariously set and one would expect the tissues of a thyroidectomised animal to be super-sensitive and not only to the action of thyroxine but to that of goitrogens also. It has been known for a long time that this is true of the thyroidectomised rat whose tissues are very hypersensitive either to thyroxine or thiouracil. Recent work shows that there is also a nervous homeostatic control of the thyroid but it is quick-acting. The present hypothesis would account for the longer-acting mechanism such as that which produced the marked bio-chemical effects in milk of the cow when daily injections of either thyroxine (Owen, 1958) or thiouracil (Chanda & Owen, 1951) are suddenly discontinued. There are then overcompensatory effects on milk yield and milk phosphatase with both these drugs as though to indicate that there is within the animal some considerable delay and some "hunting" of the homeostatic mechanism before full readjustment is achieved. If this self-rectifying action of thyroxine is indeed dependent upon the production of thiocyanate it can easily be seen that it will in turn be dependent on both the presence of sulphur atoms coming either from protein catabolism or from incoming food and on the presence of adequate amounts of vitamin B_{12} in the liver. It has been known for some years that acetonitrile (CH_3CN) can cause goitre in experimental animals. Perhaps it acts after and not before its conversion to CNS′ by the rhodanese system. As in the illustrated diagram (Fig. 1), the donor of sulphur could be cysteine or homocysteine from the catabolism of protein which results when circulating thyroxine is increased (Owen, 1958), and these S compounds could be then further catabolised to thiosulphate which is normally present in body fluids. One sulphur atom could then be transferred by rhodanese to CN supplied normally by cobalamin or abnormally from cyanogenetic substances in the food.

But perhaps Lang's rhodanese is only part of the story for very recently Kun & Fanshier (1959) have isolated from rat liver an enzyme, β-mercapto-pyruvate-trans-sulphurase, which catalyses the reactions.

(1) $SO_3'' + HS.CH_2.CO.COOH = S_2O_3'' + CH_3.CO.COOH$

Sulphite + βmercaptopyruvate = Thiosulphate + pyruvate

(2) $CN' + HS.CH_2CO.COOH = SCN' + CH_3.CO.COOH$

Cyanide + βmercaptopyruvate = Thiocyanate + pyruvate

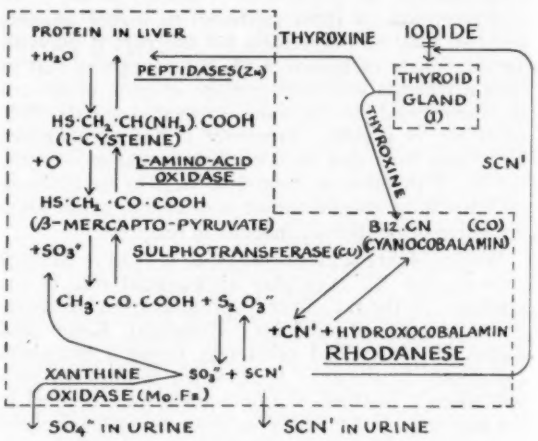


FIG. 1

Suggested Role of Trace Elements, Fe, Cu, Co, Mo, Zn and I in the Self-limiting action of the Thyroid Gland.

Katabolism of liver protein will be accelerated by thyroxine giving rise to cysteine from which α-amino-acid oxidase should generate β-mercaptopyruvate from which the recently discovered enzyme of Kun & Fanshier (1959) would produce thiosulphate and pyruvate. Lang's enzyme, rhodanese, will then transfer sulphur from thiosulphate to CN arising from cyanocobalamin. B_{12} in the form of hydroxocobalamin is then free to reform cyanocobalamin by combining with either endogenous or exogenous CN or with CN arising in the rumen, thus incorporating the cycle suggested by Wokes & Picard (1955) into the present author's scheme. The SO_3'' from the action of rhodanese is free to be recycled as shown or to be oxidised to SO_4'', the chief end-product of sulphur metabolism, by xanthine oxidase. The SCN′ from the action of rhodanese passes into the blood and so reaches the thyroid gland where it inhibits the further production of thyroxine. Further explanation is given in the test. It remains to be discovered how H_2S arising in the rumen is detoxicated and I do not know whether H_2S could be directly transformed into β-mercaptopyruvic acid or whether it is all incorporated into the red cells of the blood so constituting yet another penalty to be set against the advantages of microbial symbiosis of the ruminant and providing yet another reason for the ruminant's large requirement of cobalt. Reactions in which the trace elements are involved are shown by the symbol for the element in the diagram.

This enzyme has been shown by Kun & Fanshier (1959) to contain copper. So here we have a point of interaction of copper with cobalt. It would thus appear that a combined deficiency of copper and cobalt would not only derange the animal's haemopoetic powers but would also, by inhibiting the body's power to detoxicate endogenous and exogenous cyanide

leave that cyanide free to cause nervous lesions in the body, for cyanide in traces is known to be neuropathic (Hurst, 1942) to sheep and to be an inhibitor of cytochrome oxidase, an enzyme which contains copper (Wainio, Wende & Shimp, 1958) and which is deficient in the brains of swayback lambs (Howell & Davison, 1959). Thus possibly a similar derangement of cyanide metabolism not only accounts for the lesions of swayback in the lamb but also for the nervous symptoms often associated with pernicious anaemia in man. The objection to this theory may be raised that swayback is prevented by copper but not by cobalt, but what of the South Australian disease of sheep on pastures of the grass *Phalaris tuberosa*? There also the lesions are nervous but the specific preventive is not copper but cobalt. So far as I am aware the toxic factor in *Phalaris* has not yet been isolated. Lee, Kuchel, Good & Trowbridge (1957) found that injection of B_{12} does not relieve the condition found in sheep on *Phalaris tuberosa* pastures. Nevertheless, like the poisonous factor of the sweet pea, the toxin could easily be a cyanogenetic compound or some other substance with an electrophilic group attached.

It has always been a source of wonder to me that the sheep and cow can in the rumen reduce sulphate to the very toxic sulphide, nitrate to the very toxic nitrite and set free cyanide from glucosides. Perhaps like cyanide these are also detoxicated in the liver by enzymic systems involving cobalamin, the cobalt atom of which is known to be able to combine with all three of these toxic products. Possibly cyanide, from cyanogenetic glucosides, and sulphide from sulphate are self-defeating for, with the enzyme systems available in the liver, they could hardly coexist except at minimal concentrations.

Bibliography

BLAKELEY, R. L., and COOP, I. E. (1949), *N.Z.J. Sci. Tech.*, A., **31**, No. 3, 1.

BOXER, G. E., and RICKARDS, J. C. (1951). *Archiv. Biochem.*, **30**, 372; (1952) *Archiv. Biochem.*, **39**, 281.

CHANDA, R., and OWEN, E. C. (1951). *Biochem. J.*, **50**, 100.

DANA, E. S., and FORD, W. E. (1922). *Text book of Mineralogy, London*, Chapman & Hall.

DEWEY, D. W., LEE, H. J., and MARSTON, H. R. (1958). *Nature*, **181**, 1367.

DUNLOP, G. (1947). *Ayrshire Archaeol. & Natural History Society*, **1**, 2nd series.

HOAGLAND, M. B., KELLER, E. B., and ZAMECNIK, P. C. (1956). *J. Biol. Chem.*, **218**, 345.

HOWELL, J. MCC., and DAVISON, A. N. (1959), *Biochem. J.*, **72**, 365.

HURST, E. W. (1942). *Aust. J. Exp. Biol. med. Sci.*, **20**, 297.

KUN, E., and FANSCHIER, D. W. (1959). *Biochim. Biophys. Acta*, **33**, 26.

LEE, H. J., KUCHEL, R. E., GOOD, B. F., and TROWBRIDGE, R.F. (1957). *Aust. J. Agric. Res.*, **8**, 494; 502.

NYBERG, W. (1958). *Exp. Parasitol.*, **7**, 178.

——, GRASBECK, R., and SIPPOLA, V. (1958). *New England J. Med.*, **259**, 216.

OWEN, E. C., ELLIS, S. E., WILSON, A. L., VOSS, R., and ROBERTSON, H. (1959). To be published.

——. (1947). *J. Dairy Res.*, **15**, 142.

OWEN, E. C. (1948). *Biochem. J.*, **43**, 235.

——. (1951). *J. Dairy Res.*, **18**, 113.

——. (1954). *J. Dairy Res.*, **21**, 408.

PRIEN, E. J., and FRONDEL, C. (1947). *J. Urology*, **57**, 949.

REISNER, E. H. (1958). *Federation Proc.*, **17**, 1.

RICKES, E. L., BRINK, N. G., KONIUSZY, F. R., WOOD, T. R., and FOLKERS, K. (1948). *Science*, **107**, 396.

SMITH, E. L. (1952). *Brit. J. Nutrit.*, **6**, 295.

STEWART, J. (1951). *Brit. J. Nutrit.*, **5**, 320.

STEWART, J. (1953). *Brit. J. Nutrit.*, **7**, 231.

UNDERWOOD, E. J. (1956). *Trace Elements in Human and Animal Nutrition*, Academic Press, New York.

WAGLE, S. R., MEHTA, R., and JOHNSON, B. C. (1958). *J. biol. Chem.*, **230**, 137.

——, MEHTA, R., and JOHNSON, B. C. (1958). *Biochim. biophys. Acta*, **28**, 215.

WAINIO, W. W., WENDE, C. V., and SHIMP, N. F. (1958). *Fed. Proc.*, **17**, 330.

WILSON, A. L., *et al.* (1959). Personal communication.

WOKES, F., and PICARD, C. W. (1955). *Amer. J. Clin. Nutr.*, **3**, 383.

Molybdenum

Underwood (1956) in his book on the trace elements had considerable doubt about molybdenum as a trace element because the early experiments seemed to show that it was possible to deprive the rat of it without doing it any obvious harm even though xanthine oxidase in its intestinal mucosa almost completely vanished. Xanthine oxidase produces uric acid, the end product of nucleic acid metabolism, from xanthine or hypoxanthine. In the rat there was always the possibility that it, being a ureotelic animal (Needham, J., 1942), could bypass its nitrogenous wastes into the urea cycle of Krebs & Henzeleit when the production of uric acid as a nitrogenous end-product was partly suppressed by an imposed deficiency of molybdenum. In the chicken which in Needham's classification is uricotelic, this possibility is not open for the chicken does not produce urea. Surprisingly, the early experiments on depriving chicks of molybdenum had no worse effects than the experiments with rats. Presumably neither the rats' nor the chickens' diets were as poor in molybdenum as was supposed. Presumably, also, traces of molybdenum were avidly retained in the body. When, however, tungstate was added to the chicken's diet it competed with molybdenum for protein and produced such a thoroughgoing deficiency that death of the chickens resulted and post-mortem examinations showed accumulations of xanthine and hypoxanthine in their faeces (Higgins, Richert & Westerfield, 1956).

So far as I know a xanthine oxidase deficit has been produced by dietary means only in rats and chickens and I am not aware of any experiments of this kind on larger farm animals though there are some significant observations. Thus Ellis, Pfander, Muhrer & Pickett (1958) in a trial lasting 3 years obtained evidence of stimulation of growth by molybdenum in lambs and they attributed this to an increased ability of the lambs to digest cellulose when rumen molybdenum was adequate. Askew (1958) found calculi of xanthine in the kidneys of sheep grazing unfertilized pastures in New Zealand. After fertilization of the pastures with phosphate and lime the liver molybdenum in the lambs from the unfertilized pastures averaged 0·7 p.p.m. compared with 1·2 to 2·0 p.p.m. in the others.

Xanthine oxidase is an enzyme composed of protein, flavinadenine dinucleotide, iron and molybdate and perhaps silicate. It therefore contains riboflavin which we have been studying. Xanthine oxidase occurs in milk of certain species only (Modi, Owen & Proudfoot, 1958) and we (Crossland, Owen & Proudfoot, 1958) measured its output in milk of goats maintained

on diet very poor in riboflavin. In that experiment we had added 37 p.p.m. of sodium molybdate to the salt mixture as a precaution and there was no difference between the riboflavin-deficient goats and the riboflavin-supplemented ones in milk xanthine oxidase, a fact which we attributed to the rapid synthesis of riboflavin by the rumen bacteria. From our results only part of the riboflavin in ruminant milk is protein-bound but what percentage is bound as xanthine oxidase we do not yet know (Modi & Owen, 1956; Manson & Modi, 1957). In addition nearly all the xanthine oxidase and nearly all the molybdenum stick to the fat when milk is skimmed (Owen, unpublished; Archibald, 1951). Presumably all the molybdenum in the milk is present as xanthine oxidase for all of it (Owen unpublished) can be recovered in procedures designed to recover the non-fatty constituents from cream, and Kiermeier & Capellari (1958) have shown that molybdenum content of cow milk is proportional to the xanthine oxidase content throughout lactation. We have had 4 lactating goats on our artificial diet (Crossland *et al.* 1958) with no molybdate in the salt mixture which was modelled on that of Miller, Price & Engel (1956) so as to be poor in sulphate. Oral supplements were then tried to see which could affect the xanthine oxidase in the milk. Increases were produced by molybdate with or without riboflavin. An equimolecular mixture of sulphates of potassium and sodium caused an increase but tungstate caused a marked decrease either with or without riboflavin. After that experiment we repeated the trial with sodium tungstate several times and whether the goats were on the artificial diet or were on normal stall-feeding, tungstate markedly depressed the xanthine oxidase in the milk often to zero. Xanthine oxidase in cow milk (Modi, Owen & Proudfoot, 1959) is much higher than it is in goat milk. Nevertheless, tungstate depresses the enzyme in cow milk too, when given by mouth (Owen & Proudfoot, to be published).

Milk xanthine oxidase varies from species to species (Modi & Owen, 1956; Modi, Owen & Proudfoot, 1959). It is high in the cow, and lower but easily measurable in the sheep and goat. We found no xanthine oxidase in sow or mare milk and confirmed earlier reports that it is absent from human milk. In sow milk but not in cow or goat milk riboflavin occurs as free flavin-adenine dinucleotide (Manson & Modi, 1957). We have not studied its partition in mare's milk.

Knowing that molybdenum must occur in all greenstuff as part of the nitrate reductase system of the green parts (Nicholas and Nason, 1955) we wondered whether the absence of xanthine oxidase from the milk of non-ruminants, human, pig and horse could be due to the much bigger intake of greenstuff by the ruminants so we studied the milk of two rabbits as representing a highly herbivorous but non-ruminant species. Xanthine oxidase was easily measurable in rabbit milk.

Nor is the absence of the enzyme due to a mere deficit of molybdate in the milk for adding molybdate does not produce the enzyme in milks not containing xanthine oxidase. Moreover, tungstate added *in vitro* does not inhibit the enzyme. We have added sow milk or human milk to cow milk, but no inhibition of the xanthine oxidase activity occurs. Evidently the molybdenum metabolism of ruminants and the rabbit differs from that of non-ruminants but not in any obvious way or the pig would resemble the cow instead of differing from it. We have not yet tried the effect of copper on milk xanthine oxidase, but we shall soon be able to do so, nor have we yet tried various combinations of salts which should add other facts to the interesting observations of Dick (1956) on the role of sulphate in Mo-Cu interactions.

There is a vast literature on xanthine oxidase going back to long before the time when iron, molybdenum and flavin were found in it, but since I am dealing chiefly with trace elements I would like to conclude this section by a reference to an interesting observation of Green & Mazur (1956) that xanthine oxidase probably by producing uric acid has the power to solubilize ferritin, the insoluble ferric iron phosphate protein which occurs in the cells of the liver. Uric acid, they say, can solubilize ferritin as ferrous iron and so free it to combine with the Fe-transporting protein of the blood so explaining the body's solubilization of iron reserves after haemorrhage. In shock, they say, blood uric acid is much increased. Furthermore, uricase is a copper-containing enzyme and is sensitive to the oxygen tension in the blood, being less potent as the oxygen tension falls. If this system of Green & Mazur (1956) is in fact operative there seems no reason why it should not also explain the relief of the so-called iron absorption block in the intestinal mucosa which normally prevents too much iron from being absorbed. Ferritin and xanthine oxidase are both abundant in the intestinal epithelium so that Green & Mazur's mechanism should work there as well and should lift the block whenever haemorrhage or anaemia lowers oxygen tension.

The bacterial enzymes in rumen contents which reduce nitrate to nitrite are almost certain to be potentiated by molybdenum, for bacteria contain xanthine oxidase as well as nitrate reductase but I do not know of any work of this kind on rumen bacteria.

Finally, may I say that Dick (1956) in explaining his observation that sulphate inhibits the retention of molybdenum seems to me to discount the intervention of the large redox potential of the rumen microorganisms. In my opinion a large intake of sulphate will produce a large concentration of sulphide in the rumen with consequent precipitation of molybdenum as the very insoluble molybdenum sulphide. If molybdenum does reach the liver, Mills (1958) suggests that it will lower the sulphide oxidase activity of that organ. Obviously the interaction of Cu, Mo and SO_4'' is more complicated than was at first suspected.

Bibliography

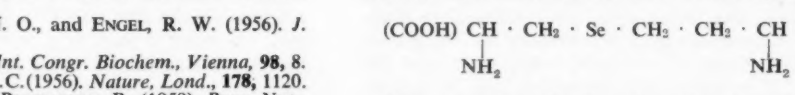
ARCHIBALD, J. G. (1951). *J. Dairy Sci.*, **34,** 1026.
ASKEW, H. O. (1958). *N.Z. J. Agric. Res.*, **1,** 447.
CROSSLAND, A., OWEN, E. C., and PROUDFOOT, F. (1958). *Brit. J. Nutrit.*, **12,** 312.
DICK, A. T. (1956). In *"Symposium on Inorganic Nitrogen Metabolism"*, Baltimore, John Hopkins Press, Eds. McElroy & Glass.
ELLIS, W. C., PFANDER, W. H., MUHRER, M. E., and PICKETT, E. E. (1958). *J. Animal Sci.*, **17,** 180.

GREEN, S., and MAZUR, A. (1956). *Science*, **124**, 1149.
HIGGINS, E. S., RICHERT, D. A., and WESTERFIELD, W. W. (1956). *J. Nutr.*, **59**, 539.
KIERMIER, F., and CAPELLARI, K. (1958). *Biochem. Zeitschrift*, **330**, 160.
MANSON, W., and MODI, V. V. (1957). *Biochim. Biophys. Acta.* **24**, 423.
MILLER, R. F., PRICE, N. O., and ENGEL, R. W. (1956). *J. Nutr.*, **60**, 539.
MILLS, C. F. (1958). *4th Int. Congr. Biochem., Vienna*, **98**, 8.
MODI, V. V., and OWEN, E. C. (1956). *Nature, Lond.*, **178**, 1120.
——, OWEN, E. C., and PROUDFOOT, R. (1959). *Proc. Nutr. Soc.*, **18**, i.
NEEDHAM, J. (1942). *Biochemistry and Morphogenesis*, Cambridge Univ. Press.
NICHOLAS, D. J. D., and *Nason*, A. (1955). *Plant Physiol.*, **30**, 135.
OWEN, E. C. Unpublished.
——, and PROUDFOOT, R. (to be published).
UNDERWOOD, E. J. (1956). *Trace Elements in Human and Animal Nutrition.*

Vitamin E and Selenium

The vitamins E (tocopherols) have turned out to be the least specific of all the vitamins whether fat— or water-soluble, for the tocopherols have proved to be replaceable in certain of their functions at any rate by various organic compounds which are known to have reducing properties such for instance as N-N' diphenyl para-phenylene-diamine, 1, 2-dihydro-2, 2, 4-trimethyl-6-ethoxy-quinoline, methylene blue, ascorbic acid or hydroquinone. Some of these substances have long been known to prevent the peroxidation of fat in stored foods and it is perhaps not surprising that they should be able to replace vitamin E, one of whose chief functions is to prevent the autoxidation of fat in the body. Perhaps they act by reducing certain pro-oxidant trace metals, such as copper, to a form in which their catalytic properties are destroyed. Recently, however, a much more surprising vitamin E substitute has been found in a variety of compounds of selenium. It has been known for a long time that selenium is present in Tenmarq wheat and it is no doubt present in other species of wheat. In Florida, though producing "alkali disease" in cattle and horses, selenium encourages the growth of certain plants such as Astragalus which therefore, because they warned stockmen of land on which cattle or horses could not safely be left to graze, became known as "indicator" plants. This problem of selenium toxicity is not just ancient history for seleniferous areas still continue to be discovered as for instance in Ireland where cattle are affected and Queensland (Knott, McCray & Hall, 1958) where horses suffer from "change-hoof" disease. That the toxic level of selenium is not very high may be judged from the fact that arsenic compounds may be used as antidotes to it. It is all the more surprising, therefore, that Schwarz and Foltz (1958) in searching for a dietary essential missing from Torula yeast, should have found it to be a selenium compound. They were looking for a Factor 3 which protects rats against necrosis of the liver which occurs when their diets contain Torula yeast as the main source of protein. They found the protective factor was preserved in an alkaline but not in an acid ash of their concentrate and found it to be a compound of selenium. Elementary selenium and many selenium compounds were ineffective but sodium selenite (Na_2SeO_3) and a wide range of other Se compounds prevented necrosis.

Among these were the Se analogues of several sulphur compounds e.g. selenocystine (isolated from wheat) selenomethionine and seleno-cystathionine:



The necrosis of the liver introduced by feeding the Torula yeast diet could be prevented also by vitamin E in which the diet was also deficient. Thus selenium must be admited as a new member of the trace elements. It is effective at a few parts per million in the diet against the appearance of lesions hitherto regarded as signs of deficency of vitamin E. It can prevent dietary liver necrosis in rats (Schwarz & Foltz, 1958) and in pigs (Stokstad, Eggert & Patterson, 1958; Grant & Thafvelin, 1958) multiple necrotic degeneration (of heart, liver, muscle and kidney) in the mouse as well as fatal exudative diathesis in the turkey (Schwarz & Foltz, 1958) and the chick (Patterson, Milstrey & Stokstad, 1957). It can also minimize the incidence of white striae in the breast muscles of chicks (Dam & Søndergaard, 1957; Søndergaard, Christensen, Dam & Prangl, 1958). But the lesions produced by diets deficient in vitamin E are so many and vary so much from species to species that a careful search is going on to see whether selenium is a complete substitute for vitamin E. It appears that it is not, but that does not mean that selenium is a trace element sailing under false colours for it is obvious that up till now no so-called vitamin E-deficient diet has been known to be free of selenium. As matters stand selenium does not prevent muscular dystrophy in the rabbit (Hove, Fry & Schwarz, 1958) or encephalomalacia in the chick (Draper, 1957). It does not prevent the depigmentation of the rat's incisor teeth, or the haemolysis of red cells in drawn blood (Gitler, Sunde & Baumann, 1958). It does not prevent muscular degeneration or deposition of ceroid pigment in adipose tissues of pigs (Grant & Thafvelin, 1958) nor does it prevent sterility with resorption of embryos in the female rat bred with normal males (Harris, Ludwig & Schwarz, 1958). Evidently there is a complicated interaction to be investigated here and I would like to know, in view of the many similarities between the symptoms of selenium deficiency and of its toxicity whether anyone has tried the effect of vitamin E on selenium poisoning. I would also like to know how Torula yeast became so deficient in a trace element in view of its having been first mass-produced for use as human food. Were the sulphates of copper and iron used in its culture solution perhaps too highly purified? Hexane-extracted brewers' yeast does have Factor 3 activity.

It is still too soon to speak about the role of selenium in enzyme actions in the body but Se is active in the rat at the level of 250 millimicrograms per rat per day and is 500 times as effective at vitamin E in preventing liver necrosis (Nutrit. Rev., 1958). Tellurium cannot be substituted for selenium. *In vitro*, Tsen & Tappel (1958) have found that selenite is a potent catalyst for oxidising glutathione and gluta-

thione has long been invoked as a pro-oxidant of fats. Selenite also catalyses the oxidation of cysteine, dihydrolipoic acid and coenzyme A. Tellurite and arsenite inhibit these selenite catalyses and, in the oxidation of glutathione (GSH), selenium diglutathione (G-S-Se-S-G) was found to be an intermediate compound. Moreover in yeast Wilson & Bandurski (1958) have found an enzyme which catalyses the exchange of sulphate for pyrophosphate in adenosine triphosphate to produce adenosine phosphosulphate. In this reaction sulphate can be replaced by selenate, chromate, molybdate or tungstate, but only selenate and sulphate give stable products. It will be interesting if such an enzyme is eventually found in animal tissues.

Bibliography

DAM, H., and SONDERGAARD, E. (1957). *Experientia*, **13**, 494.
DRAPER, H. H. (1957). *Nature*, **180**, 1419.
GITLER, C., SUNDE, M. L., and BAUMANN, C. A. (1958). *J. Nutrit.*, **65**, 397.
GRANT, C. A., and THAFVELIN, B. (1958). *Nord. Vet. Med.*, **10**, 657.
HARRIS, P. L., LUDWIG, M. I., and SCHWARZ, K. (1958). *Proc. Soc. Exp. Biol. Med.*, **97**, 686.
HOVE, E. L., FRY, G. S., and SCHWARZ, K. (1958). *Proc. Soc. Exp. Biol. Med.*, **98**, 27.
KNOTT, S. G., MCCRAY, C. W. R., and HALL, W. T. K. (1958). *Queensland J. Agric. Sci.*, **15**, 43.
Nutr. Revs. (1958) **16**, 149; 174
PATTERSON, E. L., MILSTREY, R. and STOKSTAD, E. L. R. (1957). *Proc. Soc. Exp. Biol. Med.*, *95*, 617.
SCHWARTZ, K., and FOLTZ, C. M. (1958). *J. Biol. Chem.*, **233**, 245.
SONDERGAARD, E., CHRISTENSEN, F., DAM, H., and PRANGL, I. 1958). *4th Int. Congr. Biochem. Vienna*, **92**, 8.
STOKSTAD, E. L. R., EGGERT, R., and PATTERSON, E. L. (1958). *4th Int. Congr. Biochem. Vienna*, **92**, 8.
TSEN, C. C., and TAPPEL, A. L. (1958). *J. Biol. Chem.*, **233**, 1230.
WILSON, L. G., and BANDURSKI, R. S. (1958). *J. Biol. Chem.*, **233**, 975.

Zinc

I would like to conclude this address with some remarks on the metabolism of zinc in relation to diseases of livestock. Zinc occurs in the body in greatest concentration in the skin and bones, and other important sites of concentration are the pancreas of animals and the testes of man. Milk is also rich in zinc, and colostrum particularly so. In mice deprived of colostrum death from zinc deficiency can result. Egg yolk which provides the needs for very rapid growth of the chicken is also rich in zinc. Keratinized tissues are very rich in zinc and a heavily fleeced sheep may have most of its zinc in the wool. Tissues containing melanin e.g. hair, feathers, are rich in zinc and the same applies to the choroid of the eye. A recent observation (Weitzel, *et al*, 1955) is that the tapetum of the dog and fox is richer in zinc than any other tissues so far examined and appears to consist of a complex of zinc and cysteine so arranged as to form a mirror behind the retina and so to facilitate night vision.

Underwood, in his book written as recently as 1956, doubted whether a deficiency of zinc could occur in a farm animal, but as is known, the discovery of the prevention of parakeratosis in the pig fed on dry rations was being prepared for publication at that time. Perhaps the pig's rapid rate of growth, and possibly its deficient ability to grow hair, a tissue very rich in zinc, account for the pig's very large requirement of zinc, but it still remains to be shown how the calcium in the diet exacerbates parakeratosis in the pig. I wonder if there is a protein deficiency involved as well; for an all-vegetable ration is very poor in lysine and methionine, of which a rapid grower like the pig needs a good supply, especially when it is considered that the two tissues of the body which grow the fastest are the intestinal mucosa and the skin in that order. These two tissues will have a very fast rate of turnover of amino acids for the skin and hair are continually being rubbed off by the environment and the alimentary mucosa by the passage of fibre-rich food. Probably carboxypeptidase is needed as a synthetic enzyme for the rebuilding of new protein as well as for the dismantling of the old, and carboxypeptidase is a zinc-containing enzyme (Hoch & Vallee, 1958). A difficulty of interpretation arises here for carboxypeptidase is a bovine pancreatic enzyme secreted into the pancreatic juice. I do not know whether any one has looked for it in the skin nor do I know whether the intestinal epithelium contains it or is rich in zinc.

Glutamic acid dehydrogenase in bovine liver (Adelstein & Vallee, 1958) and lactic dehydrogenase in rabbit skeletal muscle (Vallee & Wacker, 1956) contain zinc. It is easy to visualize an important role for these two enzymes in currently accepted schemes of intermediary metabolism in the animal body and the function of the zinc enzyme, carbonic anhydrase, in the erythrocytes and other tissues, such as the gastric mucosa, is evident enough. The alcohol dehydrogenase of yeast, another zinc-containing enzyme, has an obvious enough function but perhaps we shall have to wait for more biochemical data before we can assign a function to the alcohol dehydrogenase of the horses's liver, which is also a zinc protein (Theorell et al., 1955; Vallee & Hoch, 1957). Possibly this enzyme is needed by an animal such as the horse to detoxicate alcohols produced by the fermentations which occur both in its stomach (Scheunert & Kryzwanek, 1929) and in its capacious caecum and colon. We do know from the work of Robertson, Thin & Stirling (1950) that isopropyl alcohol arises from bovine rumen fermentations and that it is ketogenic. Perhaps liver alcohol dehydrogenase is another provision the excessivly herbivorous animal has to make to keep circulating ketogenic substances of microbial symbiotic origin at a minimum (Owen, 1947, 1951, 1954).

Bibliography

ADELSTEIN, S. J., and VALLEE, B. L. (1958). *J. Biol. Chem.*, **233**, 589.
HOCH, F. L., and VALLEE, B. L. (1958). *"The Metabolic Role of Zinc"*, chapter 22 in "Trace Elements". Proc. Conf. Ohio Agric. Exp. Sta., Wooster, Ohio. Eds. C. A. Lamb, O. G. Bentley, J. H. Beattie, London Acad. Press.
OWEN, E. C. (1947). *J. Dairy Res.*, **15**, 142.
——. (1951). *J. Dairy Res.*, **18**, 113.
——. (1954). *J. Dairy Res.*, **21**, 408.
ROBERTSON, A., THIN, C., and STIRLING, A. M. (1950). *Nature, London*, **166**, 954.

ROBERTSON, A., and THIN, C. (1953). *Brit. J. Nutrit.*, **7**, 181.
SCHEUNERT, A., and KRYZWANEK, F. W. (1929). *Handbuch der Ernährung und des Stoffwechsels der landwirtschaftlichen Nutztiere*, **2**, Verdaung und ausscheidung, Ed. E. Mangold, Berlin, Springer.
THEORELL, H., NYGAARD, A. P., and BONNICHSEN, R. (1955). *Acta chem. Scand.*, **9**, 1148.
THIN, C., and ROBERTSON, A. (1953). *J. Comp. Pathol.*, **63**, 184.
VALLEE, B. L., and WACKER, W. E. C. (1956). *J. Amer. Chem. Soc.*, **78**, 1771.
——, and HOCH, F. L. (1957). *J. Biol. Chem.*, **225**, 185.
WEITZEL, G., BUDDECKE, E., FRETZDORFF, A. M., STRECKER, F.J., and ROESTER, U. (1955). *Hoppe-Seyl. Z.*, **299**, 193.

THE SPEAKER'S INTRODUCTION

The Chairman, Professor E. C. Amoroso, in introducing the author, said that a very large number of elements were known to occur in the cells of the animal body. Many of them occurred in such small concentrations that the earlier workers had been impelled to refer to them as occurring only in traces. Those tracer elements, as they had subsequently come to be known, owed their importance to the part that they played in the enzyme systems of the body.

He called on Dr. Owen to present his paper.

Dr. E. C. Owen, in presenting his paper, showed some slides illustrating it.

The Opener

Mr. S. L. Hignett, who opened the discussion, congratulated Dr. Owen on a most interesting and stimulating paper; as an Association, they were indeed fortunate that Dr. Owen had agreed to make a contribution. However, Mr. Hignett said that he would be the first to admit that he had found some difficulty in preparing an adequate opening.

Whilst it was a great honour to have been asked to take part in the discussion, he not only felt, but was also in fact, quite unworthy of the occasion. He was not an enzymologist, but fortunately for him, Dr. Owen was well aware of this shortcoming, and still did not appear to take exception to his presence at the rostrum right now. His meetings with Dr. Owen had been most enjoyable and certainly, as a result, he had learned a great deal. In fact, the benefits he had derived far outweighed any concern on his part in connection with this effort. Another important reason for his difficulty was that the bulk of the material presented was so "up to the minute"—members would note that most of the references were 1958 or 1959—that the normal processes of digestion and assimilation or of assessment were not as yet fully complete.

He wished to say here and now that, in his opinion, an era was beginning when enzymologists would have the world at their feet. They would hold sway for some years to come, and he felt that they would unravel some, at least, of the mysteries associated with the common metabolic disorders in farm livestock, with immunity, with neoplasia, etc. All must appreciate the enormous magnitude of the task facing the enzymologists, since, with few possible exceptions, they could not deal only with factors influencing single enzyme systems, there being much interaction between systems. Increased interest in enzymology would most certainly mean renewed interest in the trace elements involved. This certainly gave the lie to those who had expressed the opinion that trace elements had had their day, that that particular field had been worked out.

In his paper Dr. Owen had elected to talk about enzyme systems associated with animal disease and in which cobalt, molybdenum, selenium and zinc were essential parts. Dr. Owen referred to copper and iodine when they were also implicated. One appreciated the limitations imposed by time, but he was very disappointed that manganese had not been included.

With regard to cobalt and vitamin B_{12}, to most people thinking superficially, the problem of cobalt and pine seemed simple and straightforward—a deficiency of cobalt limited the ruminal synthesis of B_{12}, lack of which caused loss of appetite and so the inevitable symptoms of pine. However, as Dr. Owen pointed out, there were anomalies with regard to the apparent species requirements of vitamin B_{12} and as yet considerable mystery as to its metabolic functions.

Dr. Owen drew attention to and explained, to a considerable extent, the finding that ruminants had an apparently much greater requirement for vitamin B_{12} than did simple stomached animals. The view that B_{12} could not be absorbed posterior to the small intestine (although based on evidence with ^{60}Co) did not fit in with the view as expressed by Underwood (1956) that a substantial proportion of human B_{12} requirement was supplied by intestinal bacterial synthesis. Also there was the curious position of the horse. Horses did not develop symptoms on pining land; they got negligible B_{12} in their food; the synthesis of B_{12} was presumably posterior to the small intestine and they did not show coprophagy—at least to the same extent as did rabbits. One could only presume that the actual requirements of B_{12} of horses were very small and similar to the levels shown by simple stomached animals and differed in degree from that shown by ruminants. In addition to the points raised by Dr. Owen in reference to the apparent high requirements of B_{12} in ruminants, it was also possible that ruminants as a class might be relatively deficient in intrinsic factor. They might need very high levels of B_{12} in the small intestine to allow absorption of physiological amounts and be similar to humans suffering from pernicious anaemia where detectable rises occurred in the vitamin B_{12} content of serum and urine when large doses —of the order of 1000 μg. B_{12}—were given by mouth without intrinsic factor. (Ross et al., 1954). It would be interesting to know the vitamin B_{12} level in the faeces of sheep and cattle affected with pine and whether enough was present to prevent B_{12} deficiency in simple stomached animals.

They were all interested in the practical problems of pine prevention and it was, to some extent, disturbing to know that in certain areas cobalt bullets, even when retained, could quickly lose their efficacy. It was to be hoped that the trials envisaged of inclusion of an acid, such as citric, would be successful. The risk would appear to be that with such inclusion the bullet itself would fairly quickly disintegrate.

With regard to the metabolic role of vitamin B_{12}—which seemed to involve protein, fat, carbohydrate and nucleic acid metabolism, in addition to the aspect of thyroid function chiefly discussed by Dr. Owen—this complexity of functions in conjunction with the extremely small requirements of B_{12} for most species suggested that the vitamin functioned catalytically as the prosthetic group of one or more enzymes. For himself and for his fellow veterinarians, he would like to get from Dr. Owen a little more clarification of the diagram in the paper. It was difficult to understand why there was no reference to thyrotrophic hormone. How did it fit into Dr. Owen's scheme? As far as he understood, the vitamin B_{12} served simply as a source of CN ions, the latter being converted to thiocyonate which in turn controlled the formation of thyroxine by the thyroid gland, i.e., thiocyanate inhibited the accumulation of iodine by the thyroid gland.

There was another point which puzzled him in this. If cyanocobalamin yielded cyanide and gave hydroxyocobalamin, then the hydroxocobalamin so formed, in reconversion back to cyanocobalamin, required cyanide from some extraneous source. In simple terms, could not the latter cyanide function equally well as a precursor of thiocyanate as cyanide from B_{12}? If so, why was B_{12} a necessary part of the cycle at all?

Lester Smith (1956) made the plausible assumption that vitamin B_{12} functioned as the prosthetic group of reducing enzymes and showed how this assumption fitted in with a wide range of experimental findings. If vitamin B_{12} did form part of a reducing system, then the antagonism between B_{12} and thyroxine could be explained in terms of oxidation—reduction potential, since iodide had to be oxidised to iodine before thyroxine synthesis could occur. Another explanation was that thyroxine increased the requirements for most, if not all, vitamins, not merely B_{12}, so that the alleged antagonism between the two might not be as significant as the author suggested.

From an immediate and more practical aspect, Dr. Owen had put forward additional very cogent reasons why stomach and intestinal worms should be kept to a minimum by a combination of good husbandry and the use of efficient anthelmintics.

Again, for obvious reasons, one had the opportunity of renewing the plea for a greater interest in geology and in

that connection it was as well to remember what Underwood (1957) said—namely, that it was the leafy part of the plant that reflected the mineral status of the soil; roots and seeds had fairly constant mineral contents. It was thus the grazing animal which was affected by local deficiencies or excesses of soil minerals.

In the case of swayback in lambs, the opener found Dr. Owen's suggestions most fascinating—particularly since copper deficiency, absolute or conditioned, was quite obviously not the only factor involved. His views immediately reminded one of hypocuprosis in cattle and its apparent variable effect on fertility. Usually low blood copper values in cows and heifers were associated with impaired breeding efficiency. On the other hand, some hypocupraemic female cattle were of normal fertility, suggesting that copper deficiency, absolute or conditioned, had no influence on breeding performance. As against that, administration of copper to the low fertility animals, in almost all cases, was followed by an immediate and dramatic rise in conception rate. Did Dr. Owen think that here there was involved an enzyme system essential for the establishment of pregnancy and requiring either copper or another factor, and that when this factor was present in adequate amounts breeding potential was normal, in spite of the animals suffering from hypocuprosis?

Referring again to the author's diagram showing the suggested role of trace elements, iron, copper, cobalt, molybdenum, zinc and iodine in the self-limiting action of the thyroid gland, it was perhaps worth mentioning that workers at the Wellcome Veterinary Research Station had noted that a marked degree of hypothyroidism in a herd was often associated with dietary phosphorus levels relatively in excess of those of calcium. To obtain more information, an experiment had been undertaken in collaboration with Dr. Ruth Allcroft. Twelve cows in the Frant herd had been divided into two groups comparable as regards age, milk yield, stage of lactation and pregnancy. Both groups had received the same basic ration but one group had been given feeding chalk so that the dietary CaO exceeded the P_2O_5. The other group had received a phosphate supplement to ensure that the dietary P_2O_5 was always in excess of CaO. The ration had been fed for a variable period up to the time of calving but never for less than ten weeks. It had been agreed beforehand that all bull calves would be sacrificed, their thyroids removed, weighed and examined both biochemically and histologically. Fortunately four cows in each group had produced bull calves. The thyroid weights of those

TABLE I

CORRELATION OF $CaO:P_2O_5$ RATIO OF DAM'S RATION WITH THYROID WEIGHT OF NEWBORN BULL CALVES

Ration	No. of animal	Weight of thyroid (gms.)	Average weight
	5	16·9	
	11	10·6	
$CaO > P_2O_5$	3	8·0	10·8
	9	7·8	
	2	31·6	
	8	29·8	
$CaO < P_2O_5$	4	15·5	21·9
	6	10·4	

calves were shown in Table I. It would be seen that there was a great deal of scatter but the "high phosphorus" cows had produced bull calves the thyroid weights of which on average were 22 g. which was just twice the average weight of thyroids taken from bull calves produced by cows in the other group. The enlarged glands on histological examination had shown evidence of marked stimulation. The results of this experiment were, they considered, sufficiently interesting to warrant repetition. However, although this observation had been made in 1953, they still had not found time for repeating it. In the text books there was frequent reference to the goitrogenic effect of calcium and yet, working with ruminants, they had been unable to detect any thyroid changes when feeding very high levels of that element. On the other hand, there was here, at least, a suggestion that phosphorus could be goitrogenic. Recent observations by Scott (1959) showed that this was also true for the cat.

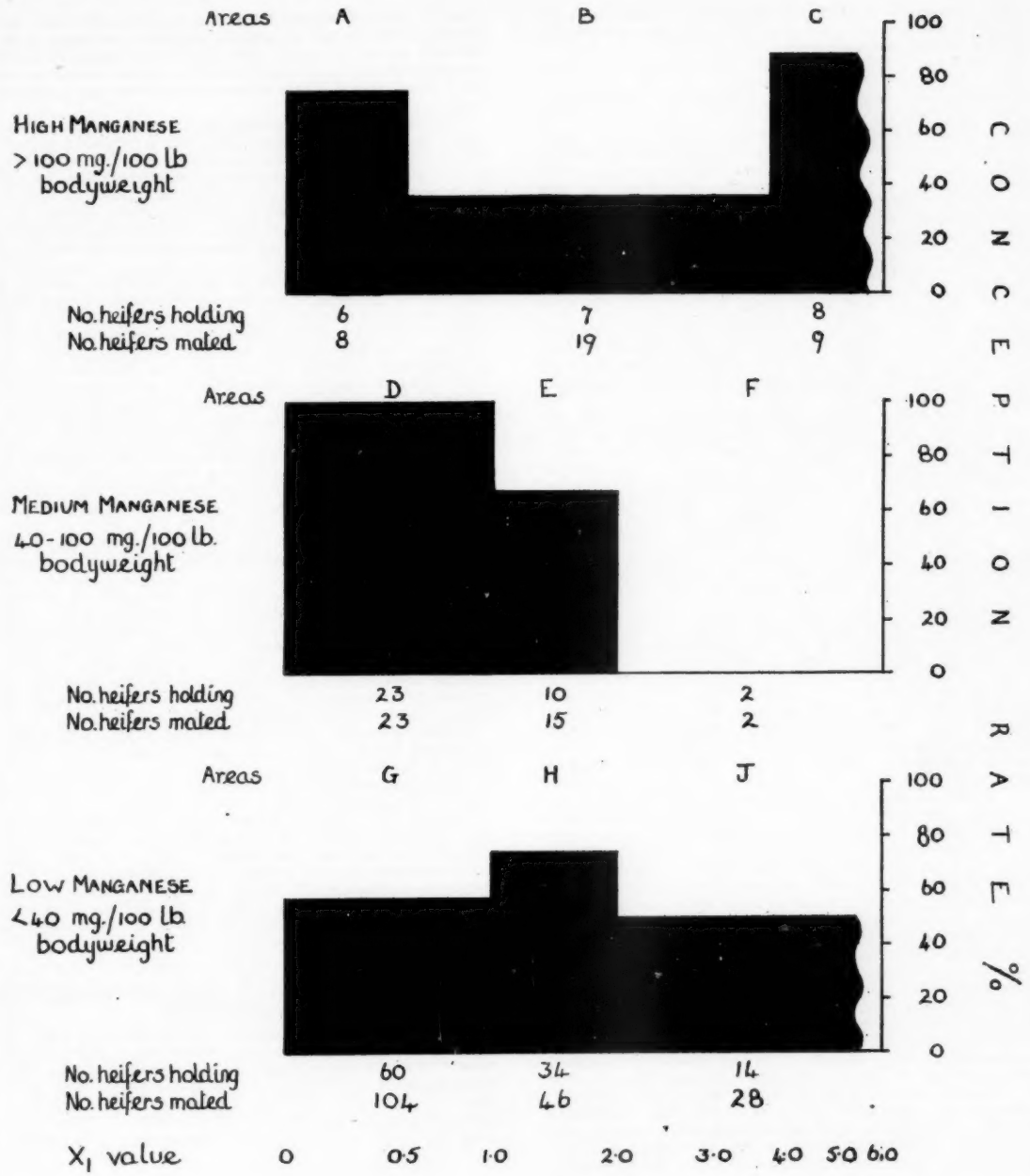
The species variation with respect to milk content of xanthine oxidase was most interesting, but the work on this aspect was still so current and incomplete that plausible explanations were not as yet forthcoming. Horses were similar to rabbits in that they were highly herbivorous, yet they fell into the same class as simple stomached animals, i.e., they had no xanthine oxidase in their milk. It did not, therefore, seem simply a question of dietary differences between species.

The competitive action of tungstate on molybdenum made one wonder whether controlled very small amounts of tungstate could be used in further studies of the copper-inorganic sulphate-molybdenum interaction, even if not as an antidote for "teart".

The work with respect to the essential nature of selenium and the part it played at trace levels in controlling some conditions previously ascribed to vitamin E deficiency was still so recent that adequate assessment was not possible. However, as recently as the 15th August, there had appeared in the correspondence columns of the Veterinary Record a letter over the signatures of Sharman, Blaxter and Wilson describing the successful use of trace levels of sodium selenite for preventing muscular dystrophy in calves.

Although the author had not referred to manganese in his paper, he (Mr. Hignett) wished to mention this trace element and to get the author's views. In planned heifer feeding experiments at the Wellcome Veterinary Research Station they had found that when dietary manganese levels were low (less than 40 mg. elemental manganese/100 lb. bodyweight) fertility was adversely affected, if calcium and phosphorus were out of balance. This effect was more marked if growth rate was high. On the other hand, with much higher dietary manganese levels (say, something in excess of 100 mg./100 lb. bodyweight) the fertility was low when calcium and phosphorus were well balanced, and high when those elements were out of balance. This was well illustrated in Fig. 1, where the dietary calcium-phosphorus status (x_1) was determined by means of the formula $\frac{(P)^2}{Ca}$ (P being the intake of elemental phosphorus in grams/100 lb. bodyweight and Ca a similar measure of calcium). Could Dr. Owen help them by suggesting an enzyme system or systems in which manganese was involved and which were essential for the establishment and maintenance of pregnancy in cattle? Phosphatase and arginase immediately came to mind. However, information they had been able to collect so far suggested that phosphatase was not the enzyme for which they were looking. Furthermore, the influence of manganese on the fertility of poultry, which did not require arginine, at least suggested that arginase might be ruled out. Furthermore, did Dr. Owen consider that an enzyme system which failed to function properly because of the absence of an essential trace element could also be influenced adversely by an excess of the same element? Certainly, since zinc toxicity was followed by a fall in cytochrome oxidase, it would appear that an excess of a trace element had the same effect on an enzyme system as did a deficiency of that element.

Finally, he uttered a word of warning to those attending a Congress in Kent at this time of the year. He had recently been reading "Rural Rides" by William Cobbett who, writing from Tunbridge Wells on Saturday, 30th August, 1823, said: "This is a great *nut* year. I saw them hanging very thick on the way-side during a great part of this day's ride; and they put me in mind of the old saying, "That a great *nut* year is a great year for that class whom the lawyers, in their Latin phrase, call the 'sons and daughters of nobody'." I once asked a farmer, who had often been overseer of the poor, whether he really thought that there was any ground for this old saying, or whether he thought it was mere banter. He said that he was sure that there were good grounds for it; and he even cited instances in proof, and mentioned one particular year, when there were four times as many of this class as ever had been born in a year in the parish before; an effect which he ascribed solely to the crop of nuts the year before."



$$X_1 = \frac{(P)^2}{(Ca)} \quad \begin{matrix} (P = g./100 \text{ lb. bodyweight}) \\ (Ca = g./100 \text{ lb. bodyweight}) \end{matrix}$$

FIG. 1
EFFECT OF DIETARY CALCIUM, PHOSPHORUS AND MANGANESE LEVELS ON FERTILITY (BASED ON FIRST MATINGS) OF EXPERIMENTAL HEIFERS

The reason for mentioning this risk in a discussion involving trace elements was the fact that nuts provided a valuable source of manganese!

References

ROSS, G. I. M., MOLLIN, D. L., COX, E. V., and UNGLEY, C. C. (1954) *Blood*, **9**, 473.
SCOTT, P. P. (1959). Paper presented at the British Small Animals Veterinary Congress, London. March, 1959.
SMITH, E. L. (1956). *Brit. Med. Bull.*, **12**, 52.
UNDERWOOD, E. J. (1956). "Trace Elements in Human and Animal Nutrition" p. 185, 1st Edit. Academic Press Inc., New York.
——. (1957). *Austr. vet. J.*, **33**, 283.

The General Discussion

Dr. W. R. Wooldridge (London): "Those who are members of the Veterinary Research Club will know that I am delighted that a paper of this character has found its way into a B.V.A. Congress, because from time to time in that Club I have mentioned and emphasised the important of cellular enzyme systems and their relationship, or possible relationship, to disease.

"Both the reader of the paper and the opener of the discussion have dwelt upon those conditions which we have not infrequently described as deficiency diseases. They have quite rightly linked these particular diseases with enzyme actions because the specific deficiencies referred to have been shown to be essential chemical elements in certain enzyme reactions. They have referred to many minerals and vitamins which are now known to be essential components either of particular enzymes themselves or the enzyme systems. In other words, the speakers have dwelt almost entirely upon what I might call the static side of enzyme reactions.

"Enzymes are the tools of many of the dynamic changes which continue in the living cells. It is these dynamic changes that are the important bases of life. Hence, whilst these changes will not occur unless the chemical molecules which make up the enzymes were not there—that is those essential minerals and elements were not present—nevertheless other substances are also essential for the changes to continue. These substances can be considered as presenting a dynamic side to this paper, whereas the paper as so far presented might be considered to deal with the static side of the problems. I wish to say a few remarks about this dynamic side.

"Firstly we must remember that enzyme changes only occur if there is a substance upon which the enzyme may act. This substance is called a substrate. Clearly then if the substrate is not there then no enzyme reaction can occur. We know of one example of this, namely, when a substrate for a particular enzyme is destroyed or eaten up by parasites, thus creating that particular deficiency. A reaction may not occur however even in the presence of the substrate because the enzyme action may be inhibited by another chemical. Two types of inhibitors are known to exist. The first is of a general character, where a substance such as toluene sits upon or becomes adsorbed to the surface of the enzyme and thereby prevents the substrate from reaching the surface. In this way the enzyme reaction is stopped. This is the type of reaction that is often thought to be the basis of certain narcotic reactions. A second inhibition, however, may be due to a chemical substance that is closely allied to the chemical nature of the substrate itself. It is known that a substrate in order to link into a particular enzyme system must have a particular chemical composition, or particular chemical groupings or chemical configuration within its molecules. This is well known but what is not quite so generally realised, despite the fact that a paper was published in 1927 by Quastel and Wooldridge, is that a whole series of similarly constituted chemical compounds could become adsorbed on to an enzyme. We showed that the substrate molecule itself was the most active of this group of compounds and that one or two other compounds in the series might be acted upon less actively. Then we showed what was more important, the fact that other compounds in the series, as they got slightly further away from the actual composition or configuration of the substrate itself, might react specifically with the enzyme molecule and completely prevent the substrate from doing likewise. In other words, the similarly constituted substrate molecules could in fact inhibit specifically a particular enzyme reaction. This was the first time this specific inhibition of an enzyme reaction had been shown although nowadays many such anti-enzymes have been discovered. Clearly such inhibitions of enzyme reactions can bring about actual illness when continued for any period of time.

"Perhaps I might again refer to some of my own earlier work. In 1938 Miss Higginbottom and I published a paper which seems to have passed unnoticed. We showed that the alpha-toxin of *Clostridium welchii* inhibited succinic oxidase in muscle tissue. This was the first time a bacterial product had been shown to inhibit a specific enzyme activity in a host tissue. Furthermore, however, I think it might be useful to indicate what thoughts we had arising from this discovery. May I quote:

> "whether the pathogenicity of *Clostridium welchii* is associated in any way with this inhibitory action upon an important respiratory mechanism of tissues is not known, but it is clear from recent progress in our knowledge of the physiological action of certain vitamines, for example, lactoflavin, ascorbic acid, etc., that the absence of factors associated with respiratory mechanisms of cells can lead to grave disturbance. Interference with enzymic systems necessary to the supply of energy to the tissues might readily lead to subnormality of function which might open the way to the development of a more active pathological condition. In this way a particular toxin might facilitate the subsequent invasion of the body by the toxogenic organism itself or by some other organism present at the time".

From this quotation you will see that the idea that interference with enzymic changes might cause serious disease was first put forward.

"I think that this is an angle that some research workers might turn their attention to in the future and try to go beyond the individual enzyme system, complicated as Dr. Owen and others are already well aware. I should like to urge this as I am confident that the dynamic side of cellular changes taken over a period of time would give results to an enquiring investigator of even greater interest to us in our understanding of the problems of pathogenicity."

Professor Seekles (Utrecht University, The Netherlands): "I should like to raise a few questions in relation to Dr. Owen's excellent paper.

"Although milk is not the most important source of vitamin B_{12}, a comparison of the B_{12} content of milk from various species seems to be interesting from the viewpoint of comparative biochemistry. A comparison of milk samples from the normal cow, goat, sheep, rat, dog, sow and human subject reveals that the vitamin B_{12} content of milk of non-ruminants is much more variable than that of the ruminants. Whereas in ruminants the range of vitamin B_{12} content of milk varies up to a maximum of 1 to 4, it runs as high as 1 to 90 in the non-ruminants.

VITAMIN B_{12} CONTENT OF MILK (MICROGRAMMES PER LITRE) FROM VARIOUS SPECIES

Cow	range	3·2 —12·4	(1 to 4)
Sheep	,,	1·0 — 2·0	(1 to 2)
Goat	,,	0·07— 0·18	(1 to 2·5)
Human subject	,,	0·10— 1·5	(1 to 15)
Sow	,,	0·03— 2·7	(1 to 90)
Rat	,,	11·0 —95·0	(1 to 9)
Dog	,,	0·70—13·0	(1 to 19)

"The considerable variations in the milk of non-ruminants are in part due to the dietary intake of the vitamin, whereas the ruminants are independent of the direct intake of vitamin B_{12}, being able to synthesise the vitamin in the rumen.

"However, when comparing concentrations of the vitamin in the milk from the species mentioned we are struck by two facts. First of all the vitamin B_{12} concentration in the milk of the rat is 4 to 8 times as high as that in the milk of the cow, the ruminant that shows the highest B_{12} content. This difference cannot be explained from a high intake only. Secondly, from the 3 species of ruminants under investigation (cows, sheep and goats), goats' milk has a vitamin B_{12} concentration that is only one fifth of the concentration of

cows' milk. Since the range of variation is small in both species, suggesting microbial balance in the rumen, the cause of this difference remains obscure.

"I am anxious to know if Dr. Owen could give an explanation for these two unexpected facts."

The Reply

Dr. E. C. Owen, in reply, said that he would like to thank Mr. Hignett for his very able discussion of the paper and for his presentation of analogous observations from other fields of animal experimentation. As he had so rightly said, the horse seemed to survive well on herbivorous diets without the aid of a rumen. Nevertheless, it was important to remember that roughage could spend a considerable time in the horse's stomach and that during this time, according to Krzywanek (1929), parts of it were fermented before being subjected to peptic digestion so that the horse's stomach, though morphologically one, was functionally dual in nature. Considerable fermentation also occurred in the caecum and colon of the horses so that B-complex vitamins could be absorbed from them, though he did not know whether B_{12} was produced or absorbed in these organs.

It would appear, from recent work in Australia, that in cobalt deficiency protoporphyrin accumulates in the sheep's blood plasma so that evidently one function of B_{12} is to potentiate in some way the enzymic combination of protoporphyrin with ferrous iron to form haeme, and thereafter haemoglobin.

Dr. Owen thought that it must now be accepted that cyanide was a neogenic metabolite in animals as it had long been known to be in plants. Also, there was cyanocobalamin which specifically combined with the intrinsic factor when given by mouth. Other cobalamins, active by injection, do not have the same absorbability in the presence of the intrinsic factor. Mr. Hignett had asked a number of questions about the involvement of the thyroid with B_{12} and about the speaker's hypothesis that B_{12} is concerned with the synthesis of thiocyanate which is a known antithyroid substance. He had asked where the pituitary came in. The answer was in the elaboration of thyrotrophin in response to nervous stimuli either from without, through the eyes, or from within, through changes of blood composition, sensed possibly by centres in the hypothalamus (Gross and Pitt-Rivers, 1952). If the thyroid was considered as having both coarse and fine adjustments his theory explained part of the coarse adjustment; the pituitary was part of the fine adjustment. Mr. Hignett had asked about the "oxidation" of iodide to iodine before its incorporation into protein. In reply, Dr. Owen asked a question himself. Could iodine not be produced in the body by the well-known reaction $Cu^{+} + 4I^{-} = Cu_2I_2 + 2I^{-}$ which requires only the simultaneous presence of cupric and iodide ions and has no need of an oxidising agent?

Apart from arginase which is activated by manganese and apart from the possible role of manganese in the nitrate reductase of rumen bacteria little was known of the adult ruminant's requirement of manganese. Neither had its zinc requirement been measured. If there was an optimum ratio of minor elements to one another it must in most diets be "right by accident" and was more likely to be an optimum ratio for the host than for the rumen bacteria in view of the experiments at the Hannah (McNaught, Owen and Smith, 1950).

Conditions in the rumen were so complex that it was impossible to predict what effect excess of one element such as zinc might have on, say, copper, particularly when it was remembered that sulphate in the rumen is readily reduced to sulphate (Lewis, 1954) and that copper is needed for the formation of cytrochrome oxidase (see references to main paper). An insoluble sulphide could easily be precipitated in the rumen and would not necessarily redissolve later in the gut.

It had long been known that, for prevention of rickets, an optimum ratio of calcium to phosphorus was needed (Owen, 1948). Mr. Hignett's results showed that this was also true for optimum fertility but he had no explanation for this very interesting observation.

As to Cobbet's observation as to the positive correlation between the nut crop and the illegitimate birth rate, it reminded the speaker of Hogben's statistic (which he quoted as an example of so-called spurious correlation) that the birth rate in England is positively correlated with the fish landings at Billingsgate. He would merely point out that nuts and fish are both sources of first-class proteins in fair quantity and that many experiments on rats have shown that production of young is proportional to the protein intake.

Dr. Wooldridge had urged the necessity for considering dynamic equilibria inside the animal body. This was what the diagram had attempted to do, all the reactions in it being either reversible or part of a cyclic process.

He was very interested in Dr. Seekles analyses of milk for vitamin B_{12} which were new to him except for the ones from ruminants. As to the differences between the cow on the one hand and the sheep and goat on the other, these might be largely dietary for experiments had shown that oral cobalt salts increase B_{12} in ewe's milk but not in cow's milk (Edwards, 1955). The comparatively huge figure for the rat's milk intrigued him for it was what he would have expected from the diagram in his article showing the antagonism of B_{12} and thyroxine. By comparison with the cow the rat had a large requirement of thyroxine to maintain its much faster metabolic rate so that, on his theory, the level of B_{12} in its tissues should be correspondingly greater as Dr. Seekles' figures showed. He wondered if the mouse and the shrew had even greater amounts of B_{12} in their milk than the rat.

References

EDWARDS, D. C. (1955). *J. Dairy Res.*, **22**, 232.

GROSS, J. and PITT-RIVERS, R. 1952). *Brit. Med. Bull.*, **8**, 136.

LEWIS, D. (1954). *Biochem. J.*, **56**, 391.

MCNAUGHT, M. L., OWEN, E. C., and SMITH, J. A. B. (1950). *Biochem. J.*, **46**, 36.

OWEN, E. C. (1952). *Brit. J. Nutrit.*, **6**, 415.

SCHEUNERT, A. and KRZYWANEK, F. W. (1929). *Die Verdauung des Pferdes* in *Handbuch der Ernahrung* USW. Ed. E. Mangold (Berlin). Springer.

The Chairman, in closing the meeting, proposed a vote of thanks to Dr. Owen for his paper, to Mr. Hignett for his opening contribution, and to all those who had taken part in the discussion.

Infertility in the Cow and Heifer

by
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There must be few veterinary surgeons whose duties take them among cattle breeders and dairy farmers who have not frequently heard opinions expressed suggesting that there is far more infertility in cattle today than there was a few years ago. Statements of this nature have regularly been heard, and read, by the author during the past 25 years. Perhaps the same type of remark was being made to an older generation of veterinary surgeons by an older generation of stockbreeders! Certainly the comments of writers on the subject during the nineteen-twenties and early nineteen-thirties would suggest that such was the case. If these statements represent the true state of affairs there must have been a time in the none-too-distant past when the infertile cow was almost unheard of. Similarly, if the implied progressive increase continues, there must surely come a time when it will be the exception rather than the rule for a heifer ever to produce a calf.

It is impossible for us to foretell the future but we can, by consulting the works of ancient writers, form some opinion as to whether there ever was a time when failure of the calf crop was not a well recognised hazard—and probably a not infrequently experienced catastrophe—of the stockman. We find evidence in the writings of the patriarchs themselves. Job (ca. B.C. 1520) writing of the unrighteous says, "Their bull gendereth, and faileth not; their cow calveth and casteth not her calf". He would hardly have made such a statement had not reproductory failure both of the bull and of the cow been reasonably well-known phenomena in his day! Moses (ca. B.C. 1491) is thought to have been more or less contemporary with the previous author. Describing the flight of Jacob from Laban he attributes the following words to Jacob: "This twenty years have I been with thee; thy ewes and thy she goats have not cast their young." Evidently breeding troubles among sheep and goats as well as infertility in cattle were no strangers to the herdsmen of Israel. One can well imagine these herdsmen attributing their troubles to the fact that the animals were having to graze the natural herbage of the wilderness; doubtless they cast longing eyes back to the old days when they pastured their herds on the lush meadows of Goshen. Today our cattlemen often deplore our improved pastures, ascribing infertility and many other ills to their use. They sigh for the good old days of natural unimproved grassland. Truly one may say "Plus ça change, plus c'est la même chose"!

Most of us can well remember the epidemics of brucellosis during the nineteen-thirties, of trichomoniasis during the 'forties, and of vibriosis during the early 'fifties. We can also without much difficulty call to mind the high incidence of anoestrum among cows and more particularly among heifers which occurred in many parts of the country during the second world war and in the immediately succeeding years. We also remember that such anoestrum was often associated with inferior feeding and with an attempt by farmers to breed from immature and under-developed animals. Even these epidemics and outbreaks were not universally severe throughout the country. Often there was a considerable difference of incidence between adjacent areas. For example, during the 'forties trichomoniasis was a major scourge throughout Shropshire and the other English Midland counties, yet it was virtually absent from the neighbouring county of Hereford and the adjoining parts of Gloucestershire. Similarly, the cattle of Herefordshire and Gloucestershire did not seem to suffer to anything like the same extent from anoestrum as did those of the Midland counties.

In the absence of definite records for the country as a whole, or even for large sections of it, it is difficult to know whether there is any real foundation for this commonly held belief that infertility has increased during recent times. One may be very wrong in imagining that what obtains in one area is in any way typical of what appertains to another area, even though these areas may be adjacent to one another. Nevertheless, it is the author's opinion that, as far as the Herefordshire-Gloucestershire area and the West Midlands are concerned, there is no reason to believe that there has been during the past two decades any progressive increase in the incidence of bovine infertility. However correct that personal assessment may be, the fact remains that infertility in cattle is sufficiently common for the veterinary surgeon in country practice to be called upon to attend a very considerable number of individual cases in the course of any one year.

Technique

The techniques employed for the examination of the female bovine genitalia, the diagnosis of pregnancy, and the treatment of infertility have been amply described by various authors (Williams, 1921; Gould, Hignett, and Steele-Bodger, 1942; Rowson, 1942; and Murray, 1943). They consist essentially of manual palpation *per rectum* of the cervix, uterus, ovarian bursae, and fallopian tubes, and visual examination of the vagina and the external *os cervicis* with the aid of a vaginal speculum. There would seem to be no point in re-describing these methods since veterinary surgeons are already familiar with them.

Examination of the Ovarian Bursae and the Fallopian Tubes

A technique for the exploration of the ovarian

bursae and the palpation of the fallopian tubes was described by Rowson (1942) and by Murray (1943). There has, unfortunately, arisen a tendency among British veterinarians to describe this as the technique "of Rowson" or "of Murray". It should not be so described. The technique was demonstrated to the present author in 1938 by one of the most eminent of veterinary gynaecologists, the late Professor Folmer Nielsen of Copenhagen. It is a modification of a technique described by Williams (1924). If a name is to be applied to it—and, in view of its importance as a diagnostic and prognostic method, this might well be thought desirable—it might appropriately be called "the Nielsen-Williams technique". Neither of the other titles should, in justice to its originators, be used. By means of the Nielsen-Williams technique the experienced veterinary gynaecologist can, with a considerable amount of confidence, express an opinion as to the normality or otherwise of the ovarian bursae and to a lesser extent of the salpinges. Dawson (1956) reporting on the clinical examination of a series of 200 barrener-discard cows (i.e. 400 ovarian bursae) states that he obtained a degree of accuracy, as proved by *post-mortem* examination, of 97·7 per cent. This degree of accuracy is certainly very high. It would probably be difficult for any but the specialised to approach it at all closely. Nevertheless, even in the hands of the specialist, a 2·3 per cent. error occurred.

It has long been felt that a test along the lines of Rubin's tubal patency test as applied to the human would prove a valuable additional aid to veterinary gynaecology. Until recently there has seemed to be little hope that such a test would materialise. The present author carried out a number of experiments in 1938 and 1939 on the uteri of cattle which had been removed immediately after slaughter. Rowson (1942) and Spriggs (1945) carried out similar experiments. All three workers came to the conclusion that Rubin's technique held little prospect of successful application to veterinary practice owing to the tendency of the bovine uterus to rupture under a comparatively low pressure of gas. Hanley (1953), however, has shewn that by a modification of Rubin's methods the test can be successfully applied not only on the *post-mortem* specimen but also in the living animal. He carried out a series of very careful investigations. As the result of these he concludes that it is usually possible to diagnose patency of at least one fallopian tube at a pressure below 80·0 mm. of mercury. He has shewn, however, that the safety margin is very low owing to the apparently very much greater risk of rupture of the uterus in the cow as compared with the human subject. The safety margin appears to be about 10·0 mm. of mercury in the bovine animal compared with about 100·0 mm. in the human female. It therefore seems likely that, as far as veterinary work is concerned, tubal insufflation will remain a technique for the specialist rather than one which can be applied with confidence in the course of general practice.

Dykstra (1955) in the course of some short notes makes reference to a new tubal patency test devised by Dr. L. E. McDonald, physiologist at Oklahoma A. & M. College. According to Dykstra this test is carried out by giving the patient an intra-peritoneal injection of 500 c.c. of a sterile starch solution. Two or three days later vaginal swabs are taken. These are placed in a small quantity of saline solution to which 4 drops of Lugol's solution of iodine have been added. A few drops of the resulting solution are examined microscopically. The finding of bluish-purple bodies under the lens indicates that at least one fallopian tube is patent. Absence of these bodies is said to prove that neither tube is patent. It is not made clear by Dykstra whether this test has been used in cattle in the field; nor is any reference given to work published by McDonald. McDonald's test would seem to be relatively simple and safe to apply. If it can be used with a reasonably high degree of accuracy in cattle it may prove a valuable diagnostic aid to the general practitioner. In the meantime more regular use should be made in general practice of the Nielsen-Williams technique which, once mastered, provides, as has been clearly proved by Dawson, a most valuable aid to diagnosis and prognosis.

Diagnosis of Pregnancy

Rowson (1952) reported that 23·3 per cent. of a series of cows culled for various reasons were found upon *post-mortem* examination to be pregnant. It is probable that had the owners of these animals known that they were pregnant a considerable number of them—possibly the majority—would have been retained at any rate until after they had produced their calves. It should be observed that Rowson was dealing with "culls". The percentage of pregnancies among "culls because of infertility" would presumably be considerably lower. Cunkelman (1948) says that of 1000 cows and heifers presented to him for sterility treatment 31·7 per cent. were found to be pregnant on examination, or were later determined to have conceived to a mating previous to the examination. This seems to be an extraordinarily high figure. It might suggest an undue anxiety on the part of the owners of these animals! However that may be, it is interesting to observe that Cunkelman noted that, of the cattle found to be pregnant at the original examination, 5·0 per cent. had shewn one or more oestra after conception. This figure approximates that finding of Hinze (1959). From a study of the breeding records of the Carnation Milk Farms herd of Holstein-Friesian cattle covering the years 1920-1950 Hinze observed that 5·6 per cent. of all cows studied had shewn one or more oestra following conception. The findings of Cunkelman and of Hinze suggest that "heat" during pregnancy is considerably commoner than had previously been suspected; cf. Williams (1921) who gives an estimate of 1·0 per cent. to 2·0 per cent. These findings confirm the experience of practitioners that it is dangerous to assume that an animal is not pregnant simply because she is presented for treatment together with the history that she has recently "been on heat". It is probably equally dangerous to assume that a cow or heifer is not pregnant simply because the owner or attendant

declares that she has never been mated. This point is particularly worth emphasis in relation to beef cattle where herd mating is most frequently the practice. The present author was consulted in 1944 regarding supposed anoestrum among a group of 12 Hereford heifers. These animals had been pastured and yarded with a young bull for some five months. The pastures and yard were situated close to the owner's house and the animals were examined morning and evening by the owner and his cattlemen. Both owner and cattlemen declared categorically that not one animal had shewn the slightest sign of "heat"; nor, according to them, had the bull ever shewn any interest in them. It was, in their view, "beyond the realms of possibility" for mating to have taken place without some of the services having been observed. So convinced were they of the thoroughness of their control that they had insisted upon hormonal treatment being administered to the heifers with a view to correcting the condition. This treatment had produced no apparent result up till the date of the author's visit. Upon examination all 12 animals were found to be pregnant and it was clear that all had been served within 6 weeks of the introduction of the bull. Subsequently 2 of the heifers aborted—possibly as a result of the energetic hormone therapy to which they had been subjected.

Donald (1943), as a result of observations on 44 cows which showed symptoms of "heat" during pregnancy, found that the incidence seemed to be independent of the age of the cow, the breed of the cow, and the sex of the foetus carried. He did find, however, that the majority of cases occurred during the first three months of pregnancy. The diagnosis of early-stage pregnancy is, therefore, frequently required of the veterinarian for his own protection if for nothing else.

Diagnosis of pregnancy in the bovine animal is made as the result of the assessment of the findings on rectal palpation of the uterine horns, middle uterine arteries (in the middle and later stages), and ovaries, together with, in some cases, an observation of the nature of the mucus at the external os of the cervix. The method of examination, together with the findings to be expected at the various stages of pregnancy, has been fully described by previous authors.

Many veterinary surgeons seem to regard the diagnosis of pregnancy of a duration of less than about 7 weeks as something which cannot be undertaken by anyone other than a specialist. It has been argued that, since the limits of variation of the diameter of the non-gravid uterine horn overlap with those of the six-weeks-gravid horn, it is not possible to state accurately whether pregnancy of less than about 7 weeks is present. That contention might be valid were the interpretation arrived at on a quantitative basis only. In view of the fact that interpretation is on a qualitative as well as a quantitative basis the objection is not valid. But, even if the basis were quantitative only, one would generally have the non-gravid horn for comparison except where pregnancy had followed multiple ovulation in which both ovaries had been involved. As it is, the "feel" of the organ is even more important, within limits, than the actual size.

It is the author's experience that at no stage is pregnancy as easy to diagnose as it is at 5 to 5½ weeks—except after the sixth month. In the majority of cases a positive diagnosis can be made at about 30 to 32 days. It is unwise to make a negative diagnosis at less than 5 weeks unless the findings in the ovary definitely preclude the possibility of pregnancy. As in all cases where the senses are involved, what is appreciable to one man may not be so to another. Nevertheless, the changes in the 5½ to 6 weeks gravid uterus are such that they should be recognisable with practice by the great majority of veterinary surgeons.

Recognition of Ovarian Structures

It is frequently very difficult to identify what one is palpating in the course of examination of the ovary per rectum. Nor is this surprising when one considers the small size of the total mass involved and the limitations on what one can appreciate by touch. The table below is included as an aid to the recognition of ovarian structures. It is essentially the same as that already published by the present author (1943) but has been amended according to the suggestion of Hancock (1948).

This table is intended as a rough guide. It does not attempt to provide a means of differentiation between the various types of "cyst" which have been shewn by research workers to occur. Dawson (1958), in particular, has demonstrated the complex nature of the ovarian cyst and reference to his work will be made later in this paper. It is, however, the present author's custom to classify the "thick-walled cyst" as a "probably-luteal cyst" and the corpus luteum which appears on palpation to contain an appreciable amount of fluid as a "possibly-cystic corpus luteum".

Corpus Luteum	Follicle	"Cyst"
(1) Solid body which does *not* fluctuate or does so only very slightly.	(1) Vesicle which fluctuates. Deep follicles do *not always* fluctuate perceptibly.	(1) Vesicle which fluctuates. Thick walled "cysts" do *not always* fluctuate perceptibly.
(2) Up to about 3 cm. in diameter.	(2) Up to 1½ cm. to 2 cm. in diameter.	(2) 2 cm. or more in diameter when ovary is palpated between about the 5th and the 17th day of dioestrum.
(3) At its stage of maximum volume often appears to occupy more space than the rest of the ovarian tissue.	(3) Except in maiden heifers *usually* appears to occupy only a small portion of the ovarian mass.	(3) May appear to involve the whole organ.
(4) Locus of ovulation can often be felt (palpated).	(4) Locus of ovulation can *not* be felt.	(4) Locus of ovulation can *not* be felt.

In certain circumstances it can be extremely difficult to differentiate between a corpus luteum and a cyst. Such differentiation may be virtually impossible as a result of one examination. Regular examinations at intervals of a few days will generally, but not always, enable one to recognise the nature of the structure. In some cases one can be certain only on *post-mortem* examination or as a result of attempted expulsion.

Etiology

The causes of female infertility in the bovine animal may be classified under 3 main headings, viz., anatomical, infectious, and functional. Infertility due to the first of these three causes, i.e., anatomical, is so unusual that one might dismiss the cases due to this cause as of no economic importance. By far the greater proportion of cases of infertility is of either infectious or functional origin.

The relative importance of infectious and functional causes is not known. Various opinions have been expressed. Gould (1942) estimated that at least 80·0 per cent. of infertility in cattle was of pathological origin. It can probably be assumed that Gould used the word "pathological" in much the same sense as the word "infectious" is used in this paper. It can also be assumed that his opinion was based, to a large extent, on his own personal observations in the South of England. Gould also stated that, in his opinion, functional infertility was likely to assume much greater importance as measures to control pathological infertility became more widely applied. The present author, working in the West Midlands at that time, would agree with Gould's estimate as far as the latter area was concerned. He is also of the opinion that Gould's prediction as far as the Herefordshire-Gloucestershire area is concerned, has indeed materialised. Today it would appear that the majority, one might say the great majority, of infertility in the last named area is of functional origin and that infertility of infectious origin is relatively unusual.

From America Hinze (1959) concludes that "cases of functional infertility are probably in the minority". He also quotes Easley, Trotter and Leonard (Aug. 1950) working in the U.S.A. as saying "of the repeat-breeding cows in a generalised survey, 75 per cent. had a low-grade infection of the uterus" and also "uterine infections are often an area problem and may vary from place to place". The American workers' figures for uterine infection agree closely with Gould's estimate of the position in England during the early 'forties. Regarding the second quotation from their paper—the present author would be in full agreement with them if the word "area" were to be replaced by the word "herd". In his experience low-grade infections of the genital tract seem to be developed with much greater frequency by the cows in some herds than by the cows in others. Generally, but not invariably, the former are in herds in which natural mating is the practice. It should be remembered, however, that in some, at any rate, there may be undetected predisposing functional abnormalities.

Anatomical Infertility

Anatomical abnormalities in the female genitalia may be the result either of developmental anomaly or of trauma. Many defects of developmental origin have been described in the literature, e.g. uterus bicornis bicollis combined with vagina subsepta (Demeter, 1926); uterus bicornis bicollis (Stuhlenmiller, 1933; Craig, 1937); cervix bifida (Williams, 1921; Stuhlenmiller, 1933; Murray, 1939). Others such as the so-called "persistent hymen" of "white heifer disease" and atresia of one or other of the uterine horns, are well-known to most practitioners. Nor must one over-look the variable anomalies presented by the "free-martin".

The "freemartin" is, of course, permanently sterile and, although many of these animals are readily recognisable by their external appearance, some are not. For that reason it is important that the veterinarian should be on the outlook for such cases among any bunch of heifers presented for treatment for anoestrum. Of the other anomalies mentioned only the "persistent hymen of white heifer disease" and atresia of one or other of the uterine horns are known to interfere with fertility. Even cervix bifida which, though one would not expect it to interfere with conception, might be expected to prove an obstacle to parturition, does not, in the experience of the author, generally do so.

The most common abnormalities resulting from trauma—such trauma almost invariably occurring during parturition—are artificial cloaca, hypertrophy and sometimes stenosis of the cervix, and stenosis of the vagina.

Infectious Infertility

Apart from brucellosis, trichomoniasis, and vibriosis, and possibly certain other epidemic diseases which result in infertility on a herd basis, it is recognised that infertility may result from infections, generally of a low-grade probably non-specific type, affecting various portions of the genital tract. It has long been assumed that these non-specific infections are usually of an ascending nature. Spriggs (1945) and Dawson (1956) produce evidence which may lend some support to this view. The former found that of 50 cases shewing "ovarian adhesions" diagnosed in clinical examination 25 (50·0 per cent.) had a history of retention of the afterbirth or of concomitant infection in the uterus. Dawson, as the result of *post-mortem* examination of 200 barrener-discard cows, shewed that in 36·0 per cent. of cases which displayed bursitis-salpingitis there was a history of retention of the afterbirth or of other abnormal *post partum* conditions in the uterus following the immediately previous calving. In only 8·0 per cent. of non-bursitis-salpingitis cases was there any such history. It appears from Dawson's work that not only are infections of the anterior portions of the genital tract frequently the result of ascending infection but that, as has long been suspected, retention of the afterbirth is liable to jeopardise the future fertility of the cow.

Low-grade infections of the genital tract may result

in vaginitis, cervicitis, endometritis, pyometra, and salpingitis often associated with ovarian bursitis. The last condition is frequently encountered in the absence of "at present" clinically detectible diseased conditions in the more posterior portions of the genitalia.

Vaginitis: The significance of vaginitis *per se* as a factor in bovine infertility has been the subject of much controversy. Some veterinarians consider it to be a causal factor. Others tend to disregard it. Trautman (1954) reports the finding of granular vaginitis in 51·0 per cent. of 4,616 cows at the time of insemination (44·0 per cent. "mild" and 7·0 per cent. "severe"). The cows with "mild" symptoms had a fertility rate 3·2 per cent. lower than had non-affected cows, and those with "severe" symptoms 10·8 per cent. lower than had the non-affected. He says that, in spite of there being a slight tendency for decreased fertility, even the severe cases had a fertility rate (under artificial insemination) of 58·1 per cent. which he regarded as fairly satisfactory. Trautman's experience suggests that vaginitis may be a cause of infertility. One must not attach a greater significance to his findings than that. The diagnosis was made by lay inseminators and, though there is no reason to doubt that it was substantially correct, one might question the layman's ability to recognise any but grosser pathological changes in the other parts of the genitalia. It is not clear, therefore, what proportion were cases of vaginitis *per se*. Wright (1945) is of the opinion that vaginitis is not a common cause of infertility. The present writer's experience agrees with Wright's. He has never recognised vaginitis associated with infertility except where there have been concomitant pathological changes in the uterus. (That, of course, is not the same thing as saying that vaginitis *per se* has not been associated with infertility). He has also frequently observed what he believes to be the condition described as "granular vaginitis" widespread among cows and heifers in herds with no history of breeding troubles.

Cervicitis: As in the case of vaginitis there seems to be considerable difference of opinion among veterinarians as to the importance of cervicitis. It is regarded by some as the cause of infertility. Wright (1945) does not consider it an important cause. With this opinion the present author is in agreement.

Endometritis: It is generally considered that endometritis is an important factor in the causation of breeding failure in the cow. When and where infertility of an infectious type is prevalent endometritis is probably the commonest clinically recognisable lesion encountered. It is characterised by enlargement of the uterus, the enlargement being the result of thickening of the walls. It is frequently associated with a mucopurulent discharge from the *os cervicis* which is variable in amount. In some cases which are described clinically as endometritis there is no discharge. It may be that these are not, in reality, cases of endometritis. It might be better simply to describe them as cases of uterine hypertrophy, cf. Smythe (1943). The present author has considered these to be either cases of endometritis in which the discharge has not as yet appeared or else cases which have shewn discharge previously, the discharge having now ceased. Some cases exhibit the discharge only after rectal manipulation. In the classical case the discharge is readily seen, though it may be only intermittently present. Bacteriological examination of the discharge may prove sterile or may reveal various bacterial species; of these *Corynebacterium pyogenes* is probably the one most frequently recovered.

Outbreaks of trichmoniasis are usually associated with a high incidence of endometritis and of pyometra.

The decline in the incidence of endometritis in the author's district appears to have been quite marked during the past 10 years or so. This decline may well be attributable to two principal factors, viz. (1) the reduction in the opportunity for spread of genital infections owing to the great increase in the practice of breeding by the artificial insemination technique, and (2) the introduction of more efficient medicaments for the treatment of cases of retention of the afterbirth.

Pyometra: This condition is characterised by the presence in the uterus of a variable amount of pus or of pus-like material. It occurs under the same conditions as endometritis. The size of the uterus may vary from a little larger than that of a cow about 5 weeks pregnant to that of one about 10 to 12 weeks pregnant, or even larger. There may or may not be persistence of the corpus luteum. There is frequently a mucopurulent discharge, often copious though it is often only intermittent. Differentiation between the pyometrous and the gravid uterus can sometimes be achieved only with difficulty. The salient features of the differential diagnosis have been described by writers on gynaecological technique. They can be appreciated with confidence only after considerable experience has been obtained.

Salpingitis and Ovarian Bursitis. It is commonly believed that salpingitis and ovarian bursitis are usually the result of an ascending rather than a descending infection. The evidence of Dawson (1956; 1958) and to a lesser degree that of Spriggs (1945) suggests that this is probably correct.

When salpingitis is bilateral and results in blockage of the tubes infertility is complete and probably permanent. Where only one tube is involved the chances of the animals conceiving must be reduced to somewhere in the region of 50·0 per cent. of the normal.

Ovarian bursitis, if severe, may well interfere with ovulation and with passage of the ovum into the *ostium abdominale* of the fallopian tube. It is probable, however, that the real clinical significance of ovarian bursitis is that it indicates a possible present or an almost certain previous infection or inflammation of the fallopian tube. It is in this light that the finding of ovarian bursitis should be interpreted.

Functional Infertility

Infertility of functional origin may be the result of nutritional imbalance or of an upset in the ovarian system. Examples of the former are (a) interference with the normal phosphorus-calcium ratio (cf. Hignett & Hignett, 1953) and (b) possibly deficiency of

vitamin A or vitamin E. They are most likely to be associated with a problem of herd infertility and are therefore outwith the scope of this paper.

As far as infertility in the individual animal is concerned upsets in the ovarian system play an extremely important role. The ovary is concerned in two distinct cycles, viz., a reproductive cycle and a hormonal cycle neither of which is necessarily dependent on the other. Normally in the cow the two cycles are synchronised in such a way that ovulation (part of the reproductive cycle) occurs on the day following oestrum (part of the hormonal cycle). The commencement of oestrum precedes ovulation by about 36-40 hours. Any failure of this synchronisation leads to a decrease in fertility. When it is remembered that, in addition to having to fulfil this dual role, the ovary is itself part of a large endocrine system controlled by the anterior pituitary gland one wonders how it is that reproduction is generally brought to a successful conclusion. That it is not invariably so is not at all surprising.

Certain abnormalities in the ovarian system are recognised to be common causes of infertility in the cow, viz.: anoestrum, suboestrum, and "cystic disease of the ovaries". Others are suspected of playing a not insignificant part, e.g., delayed ovulation and persistence, with or without cystic degeneration, of the corpus luteum.

Anoestrum and Suboestrum: The terms "anoestrum" and "suboestrum" are used according to the definition of Murray (1942). "Anoestrum" is characterised by the absence of both the reproductive and the hormonal cycles. In "suboestrum" the reproductive cycle appears clinically to be normal while the hormonal cycle, as judged by the exhibition of visible oestrum, is depressed.

Anoestrum is commonly encountered in heifers and cows under conditions of stress, e.g., due to inadequate nutrition, heavy milk production, or debility from disease. Suboestrum may be found both in heifers and in cows, in the latter commonly at the peak of lactation. Suggestions as to possible reasons for the occurrence of anoestrum and suboestrum have already been put forward (Murray, 1942). It is the author's belief that, as a result of stress, animals may sometimes exhibit firstly suboestrum and then, if the stress continues, anoestrum. It is not suggested, however, that anoestrum is necessarily preceded by a period of suboestrum.

Cystic Disease of the Ovaries: Cystic diseases of the ovaries is a commonly encountered cause of infertility. The extremely complex nature of this condition has long been suspected by clinicians. Research workers during the past few years have shewn that it is even more complex than had been suspected.

Various estimates have been made as to the frequency of the condition. Dawson (1957) quotes one worker in South Germany as having found on *post-mortem* examination 5·0 per cent. of sterile cows and 16·0 per cent. of sterile heifers slaughtered affected, another worker in South Germany as finding 14·0 per cent. of sterile cows culled affected, a Japanese worker as finding 9·7 per cent. of all barrener-culls affected, and an American worker as finding over a 10-year period that 7·0 per cent. of cows and 3·4 per cent. of heifers exhibited cysts in any one year and that 19·0 per cent. of all cows developed them sooner or later. Dawson himself (1958) found an incidence of 25·4 per cent. among 189 discard barrener cows—a very high figure. He found no evidence to suggest a difference in breed incidence as between Guernseys and others. Animals were usually affected in their third or fourth lactation. This was when Garm, quoted by Dawson, found the disease to occur most frequently among his Friesians and a year later than among his Red Swedish Cattle. Dawson quotes evidence suggesting that, as is probably the experience of most general practitioners, the conditions seems to have a direct co-relation with high milk yield. Cunkelman (1948) diagnosed on original examination "cystic ovaries" in 8·78 per cent. of the cows and heifers which he found to be actually non-pregnant. Hinze (1959) found that "cystic ovaries and short oestral cycles directly delayed conception in 29·4 per cent. of all cows in the herd at some time during their life time".

It will be seen that most estimates vary between 5·0 per cent. and 16·0 per cent. of "barrener-culls". Dawson's own findings suggest a much higher incidence among barrener-culls in England. It seems possible that Dawson's findings were obtained from an unintentionally selective sample. Hinze's findings, since they include "short oestral cycles", are not directly comparable.

The present author would go no further than to say that cystic disease of the ovaries is, in his experience, common among dairy cows and considerably less common among cows of the beef breeds with which he deals.

Delayed Ovulation: Delayed ovulation has been shewn to occur. More frequently it has been suspected, in the absence of any demonstrable cause, as a cause of infertility. It has been thought to occur as a result of manganese deficiency and as a result of upset of the phosphorus-calcium ratio. Hinze (1959) considers that it is a cause of infertility but seems to believe that it is of limited importance.

Persistence of the Corpus Luteum: Persistence of the corpus luteum has long been described as a cause of failure of the cow to show signs of oestrum. Clinicians have spoken of corpora lutea persisting over long periods. Smythe (1943), Hancock (1948), and Dawson (1959) all describe cystic degeneration of the corpus luteum together with a prolongation of its life in some cases and a consequent suppression of oestrum. They associate this condition with infertility. It has not been the author's experience to recognise, in the absence of a uterine deciduoma, persistence of the corpus luteum as a cause of suppression of oestrum for more than a few days beyond the time when it might have been expected to occur. He believes that in the absence of a deciduoma true persistence of the corpus luteum for more than a few days is a rare occurrence. Some corpora lutea certainly do seem to contain a clinically appreciable amount of fluid and it may be that they tend to have

a life longer than normal. It is interesting to note that Hinze (1959) records that he found that irregular oestral cycles of longer than usual duration were not associated with a drop in fertility to below the herd average. This finding in somewhat surprising.

Suggested Therapy and Prognosis

When it is remembered that most infertile cows are temporarily, rather than permanently, infertile, it will be appreciated that it is extremely dfficult for the general practitioner to be definite with regard to the beneficial effects he may claim for any form of therapy which he may apply or prescribe. Unless he is strong-minded and independent enough to keep a large number of his cases as untreated controls his claims for any therapeutic measure are a matter of opinion rather than of proven fact. An experiment carried out by Dodson (1958) may be cited to illustrate this.

Dodson, when trying out the efficacy of certain treatments on 38 infertile cows and heifers, left 100 untreated controls for observation. He found that 57·0 per cent. of these controls became pregnant within 125 days of the 85th day *post partum*. This means to say that, if infertile cattle are presented for treatment soon after the 85th day *post partum*, one may expect that over half of them will become pregnant. This should occur even if one simply prescribes that each be given a table-spoonful of coloured water once daily for any specific period of time, or if one instructs the owner that a white circle is to be painted on their backs on the day of the full moon and a crescent when that heavenly body attains its first and third quarters. Dodson suggests that the only true criterion of the efficacy of any treatment is the amount by which the lag phase (the period between the 85th day *post partum* and the day on which conception occurs) is shortened.

It seems certain that many of our claims for success in the treatment of infertility are made as the result of somewhat slender evidence. Our differential diagnosis is often faulty and based on a very limited number of examinations of the case. Frequently, owing to the rush of general practice, we reach a diagnosis as a result of one single examination and we prescribe rather empirically, a line of treatment. Nevertheless, unless our treatment is actually harmful more than half of our cases treated early will "respond", most of them fairly rapidly. As long as we do not deceive ourselves into arguing along the lines *post hoc ergo propter hoc* no great harm is probably done. The position is different when one comes to deal with long-standing cases. Here, the chances of "response" to indiscriminate therapy are not so good and a more careful diagnosis and assessment of the probable prognosis are required.

Anatomical Defects of Developmental Origin: Some practitioners have recorded success in the treatment of "white heifer disease" by breaking down the so-called "persistent hymen". As it is generally considered inadvisable to breed from these animals the operation is not very often attempted. Since the second world war there has been a marked decrease in the number of shorthorn cattle kept in the author's district and today "white heifer disease" is very seldom encountered by him. Where it does occur slaughter is advised.

Atresia of one uterine horn is sometimes met with and it has been observed that cattle shewing this complaint may breed. As would be expected, these animals are not good breeding risks, owing to the fact that for physical reasons about half the oestral periods must prove sterile. Slaughter is generally advised in view of the possibly hereditary nature of the complaint.

Anatomical Defects of Traumatic Origin: Cases of artificial cloaca are generally, if not invariably, affected with a secondary ballooning of the cranial portion of the vagina. This ballooned portion of the vagina usually carries a pool of evil-smelling fluid, the result of contamination of the vaginal mucus with faecal material from the rectum. Treatment consists in advising the owner to douche the vagina with normal saline solution or with weak bicarbonate of soda solution every 3 or 4 days, and particularly during oestral periods, with a view to obtaining as sanitary conditions as possible for service. Service by intra-uterine artificial insemination as soon after calving as possible is also recommended on the assumption that, the longer the condition has been present, the greater will be the extent of contamination of the internal genitalia from the rectum. There is frequently involvement of the uterus and in such cases the endometritis ensuing should receive appropriate treatment. Cows shewing artificial cloaca together with gross contamination of the cranial end of the vagina have been encountered which conceived following natural mating though artificial insemination had failed on several occasions. The prognosis given in cases of artificial cloaca is guarded.

No attempt is made to treat stenosis of the vagina. Slaughter is advised because of the possibility of dystokia and further damage at future calving.

Hypertrophy of the cervix is frequently seen particularly among old Hereford cows. It is doubtful whether it interferes with the fertility of these animals. Often it is accompanied by eversion of the cervix and vagina. When this occurs repeatedly in the non-pregnant cow slaughter is resorted to. Amputation of the cervix has been undertaken but it is thought to be generally not warranted in beef cattle.

Vaginitis: It is now many years since the author has encountered any number of cases of vaginitis associated with infertility. When he did, these cases seemed to be accompanied by endometritis. The only treatment given was that some of the solution used for intra-uterine injection was injected into the vagina. This procedure was carried out as a routine. Lugol's solution (1 part iodine, 3 parts potassium iodide, and 400 parts of water) was used. The reason for this douching of the vagina was rather to induce straining, on the assumption that this straining might help in the removal of the discharge from the uterus, than with any idea of treating vaginitis.

Endometritis: The treatment of endometritis by the intra-uterine injection of Lugol's solution of iodine has been a common practice for many years. It

generally seems to be a satisfactory treatment. There has been division of opinion as to how the treatment works. Some veterinarians favour the theory that the iodine is used for its antiseptic properties, others that it is used as a mild counter-irritant, and others believe that it corrects an iodine shortage. It is possible that all three actions may contribute. The author has used it in the belief that the counter-irritant, or stimulant, action has been the one principally involved. The solution commonly recommended consists of 1 part of iodine, 3 parts of potassium iodide, and 1000 parts of water. It has been the author's practice to use a solution considerably stronger than this, viz.: 1 part of iodine, 3 parts of potassium iodide and 400 parts of water. Using this solution he has injected 6 to 12 ounces, depending upon the size of the patient, roughly equally divided between each uterine horn and the vagina. This treatment has been repeated once or twice at intervals of about 7 days. He has also, in some cases where there has been a more profuse discharge, used a similar volume of equal parts of 1 : 1000 acriflavine solution and glycerine (or saturated solution of magnesium sulphate) with a view to increasing osmosis from the uterine walls. When the latter treatment has been employed he has made a habit of giving a final irrigation with Lugol's solution because of its stimulent effect. For the last few years it has been his custom to use a solution of 500 mg. chlortetracycline in place of the saturated solution of magnesium sulphate and acriflavine in such cases.

The above treatments are applied and then service or insemination allowed at the next ensuing oestrum.

Where uterine enlargement has not been marked he has frequently used the technique of intra-uterine irrigation 24-48 hours after service or insemination. Lugol's solution has been used empirically for its stimulant effect.

Many cattle conceive following these treatments but no controls have been kept. It is the author's impression, however, that more have conceived than he would expect to have conceived had no treatment been employed.

Hinze (1959) with a view to overcoming possible low-grade uterine infection treated 130 cows by intra-uterine injection. These cows had been served on an average 3·3 times before treatment. They were each given 600,000 units of penicillin-6-procaine, 200,000 units of penicillin-6-sodium buffered with sodium citrate, and 2·0 gm. of dihydrostreptomycin by intra-uterine injection 48 hours after mating. Hinze found that 50·0 per cent. conceived at that mating and that a further 18·0 per cent. conceived at the next "heat" without further treatment.

Lindley (1954) using penicillin-sodium or penicillin-potassium 200,000-500,000 units and 1.0 gm. of streptomycin sulphate 24-48 hours after service published results suggesting that this treatment is probably beneficial. He found that when the penicillin-streptomycin mixture was injected in 50 c.c. of sterile saline solution 64·63 per cent. of 82 infertile cows conceived to the immediately preceding service. When it was injected in the same quantity of sterile distilled water 50·98 per cent. of 51 cows conceived. Only 33·33 per cent. of 48 controls conceived. He also treated 9 cows with the same penicillin-streptomycin mixture combined with 50 c.c. of 12·0 per cent. sulphapyridine solution. Of these 7 conceived to the immediately preceding service. It looks as if penicillin and streptomycin combined and used as an intra-uterine injection 24-48 hours after service is beneficial in the treatment of infertility probably associated with low-grade uterine infection. There is slight evidence that better results may be expected, if these antibiotics are combined with sulphapyridine.

It seems probable that endometritis is frequently the result of infection in the immediate *post-partum* period particularly if the fœtal membranes are retained. Working on that assumption it is the author's practice to attempt to prevent such infection becoming established. He believes that cases of retained afterbirth should receive prompt attention. He removes the membranes as soon as it is possible to do so without the use of force and preferably not later than 3 days *post partum*. Dressings of 50-100 mg. of stilboestrol and 500-1000 mg. of chlortetracycline are introduced into the uterus. The usual application is 50 mg. of stilboestrol and 500 mg. of chlortetracycline. These applications are repeated after 48 hours and continued every 2 or 3 days until the uterus appears to be clinically normal. Where more than 2 dressings are required and where the lochia appear to contain a considerable proportion of "runny" mucus the stilboestrol is discontinued. If the fœtal membranes are readily removed and if there appears to be a relatively healthy uterus, 100 mg. of stilboestrol with no chlortetracycline is applied. When it is not found possible to remove the fœtal membranes on the first occasion dressings are applied generally consisting of 100 mg. of stilboestrol combined with 500 mg. of chlortetracycline. Visits are made every second day and the membranes are removed as soon as is posible. The fœtal membranes seem to have a tendency to become somewhat gelatinous following the application of 100 mg. of stilboestrol to the uterus. This gelatinisation is an undesirable feature as it renders the membranes more difficult to handle.

The author believes that by pursuing an active antibiotic policy with regard to cases of retained afterbirth he has helped to reduce the number of cases of endometritis in his practice. In this connection it is interesting to note the results recorded by Vigue, Fitzgerald, and Castrucci (1959). These workers in a series of 530 cattle kept half as controls and treated the other half by routine application of nitrofurazone pessaries into the vagina. The pessaries were applied twice weekly for a few weeks after calving in the case of the cows, and in the case of the heifers for a month before mating. In both cases treatment continued until after conception occurred. It was found that those treated with nitrofurazone in this way required 1·88 services per conception whereas the controls required 2·85 services. Likewise, the number of days between calvings was 385 among the treated and 447 among the controls. They also report that, while there was no significant difference in the nature

of the bacterial flora of the vagina after treatment, the actual number of bacteria recovered was reduced.

It should be borne in mind that routine immediate *post partum* antibiotic intra-uterine therapy may not be without danger. Hinze (1959) found that 75 cows treated with oxytetracycline and 74 cows treated with a mixture of neomycin, urea, boric acid, and sulphathiazole bred less efficiently than did 79 controls. He found the conception rates to be as follows:— oxytetracycline group 2·24, neomycin mixture group 2·56, and control group 1·67. It is difficult to explain why this result should have been obtained. One notes, however, that in the case of the oxytetracycline group at any rate, a fairly large dose (2 gm.) was used. Nevertheless, with the proviso that large doses of oxytetracycline and of neomycin may be contra-indicated, the present author believes that immediate *post partum* intra-uterine antibiotic therapy is to be recommended where there is a danger of low grade infection of the internal genitalia.

The prognosis given in cases of endometritis in which there is normal ovarian activity should be excellent. In cases where the ovarian cycle is abnormal or is absent it should be more guarded. In such cases it is unlikely that the uterus will be restored to health as long as the ovary is not undergoing hormonal cycle. Attention to the ovarian abnormality should in such cases, take precedence over the treatment of endometritis.

Pyometra: The aim in the treatment of cases of pyometra is two-fold, viz.: (1) to cause the uterus to expel its pus-like contents and to remain empty and (2) to bring under control any infection which may be present. Of these, the second is relatively easily achieved provided that the first objective can be attained. It is often fairly easy to induce the pyometrous uterus to expel its contents but there is a marked tendency for it to relapse into its former state unless the ovaries can be induced to undergo a normal hormonal cycle.

In the majority of cases of pyometra examination of the ovaries reveals them to be "cystic", clinically anoestrous, or, less frequently, to contain a corpus luteum not associated with a normal reproductive cycle. It may well be that if one were to examine the ovaries of those diagnosed "clinically anoestrous" or containing a corpus luteum one would find evidence of cystic degeneration in these also.

It has been shewn experimentally that over-oestrogenisation will render the mouse's uterus pyometrous and it has been the experience of many workers, including the author, that prolonged administration of oestrogens to the bovine animal tends to produce a thinning of the wall of the uterus and a pyometrous condition. These findings, together with the fact that it is unusual for pyometra to occur in association with a normal oestral cycle, suggest that the probable primary cause of pyometra in the bovine animal is an ovarian upset accompanied at some stage by excess oestrogen production. That is not to say that the pyometrous condition will resolve immediately the excess oestrogen production ceases. Indeed, the fact that many pyometrous cows will discharge the uterine contents when a dose of about 25 mg. of stilboestrol dipropionate intramuscularly followed 48-72 hours later by 7-10 c.c. of pituitrin is given, though they will fail to do so if pituitrin only is administered, suggests that such is not the case. It seems logical to suppose that many of these pyometrous cows which on rectal examination fail to reveal "cysts" have probably gone through a stage when "cysts", associated with excess oestrogen production, were being produced. In this connection it is interesting to note the concept reached by Dawson (1957) viz.: "a typical case of cystic ovarian disease goes through a phase of active nymphomania during which cysts succeed one another in attaining an active peak of oestrogen secretion and then degenerate, though they may persist. Later quiescence may supervene, when all the cysts, large and persistent though they may be, are more or less inactive". Is it possible that, in some cases, the amount of oestrogen production of the early active cysts may have been below the threshold required to produce subjective signs of oestrum but may yet have been sufficiently great and of sufficiently prolonged duration to affect the uterus? Or, alternatively, is it possible that the amount of oestrogen production was so high that the animal did not react by shewing subjective signs of oestrum? If either of these is physiologically possible it might explain the origin of these "cystless" cases of pyometra. Whatever the explanation is, the fact remains that, in the treatment of pyometra, prime consideration must be given to the probable hormonal state of the patient.

In cases which shew symptoms of oestrum, evacuation of the uterine contents may be induced by the administration of posterior pituitary extract. The posterior pituitary extract is probably best administered about the time that oestral symptoms are shewn. Where no oestral symptoms are shewn a dose of stilboestrol dipropionate of the order of 25 mg. may be given intra-muscularly followed in 48-72 hours by a dose of 7-10 c.c. of pituitrin. Where cysts are palpable an attempt to restore normal ovarian function should be made by rupturing these, if necessary at least twice at an interval of 8-10 days, before any resort is made to therapy involving the use of oestrogens. Where a corpus luteum is present manual expression may be attempted. This is likely to prove difficult and resort may be made to intramuscular injection of stilboestral dipropionate in doses of up to 30 mg. A dose of pituitrin may then be given when oestrum supervenes or when the amount of discharge is seen to increase noticeably.

Although intra-uterine injections are not generally to be recommended in cases of pyometra the author has found that the careful administration of 3-4 oz. of strong Lugol's solution (1 : 3: 200 or even 1 : 3: 100) is frequently beneficial in inducing evacuation of the uterus provided that the cervix is open. This procedure, however, must be undertaken with great care not to cause trauma to the wall of the uterus or to the cervix.

Once the uterus has discharged its contents the author administers 500-1000 mg. of chlortetra-

cycline every 3 or 4 days as long as there is marked discharge. He believes that it is beneficial to conclude treatment by injecting Lugol's solution (1:3:400) for its stimulant effect.

The prognosis in cases of pyometra is good unless the cases are of long standing and associated with "clinically inactive" or cystic ovaries. In such cases a doubtful prognosis should be given.

Salpingitis and Ovarian Bursitis: There is no known method of treatment which can with confidence be applied to cases of salpingitis. It is possible that some cases may resolve without treatment but it is probable that cases which are presented for infertility treatment and which shew direct evidence of salpingitis by the Nielsen-Williams technique have already reached a stage of chronicity from which it is unlikely that spontaneous recovery will occur. Therefore, cases of clinically diagnosable bilateral salpingitis must be regarded from a practical point of view as probably permanently infertile.

Where only one tube is found to be clinically affected a slightly more hopeful prognosis may be given. It should nevertheless be borne in mind that salpingitis and probable lack of function, if not of patency, may exist in the absence of detectable enlargement. Thus Dawson (1958) found 18·25 per cent. of such cases. Therefore, even where detectable salpingitis is only unilateral prognosis is not good.

In view of the probability that most cases of ovarian bursitis are the result of an ascending infection one must suspect that there has at one time existed salpingitis in the tube of the bursa affected. For that reason, where ovarian bursitis is marked and bilateral, prognosis should be grave. Where it is unilateral the prospects of the affected animal breeding are considerably better. Nor should it be overlooked that pregnancy has been shewn by Rowson (1942) to occur in the uterine horn of the affected side of some cases of ovarian bursitis.

It is the author's custom to break down bursal adhesions where they exist and are not too severe in the hope that he may remove mechanical obstructions to the entry of the ovum into the *ostium abdominale* of the fallopian tube. A very doubtful prognosis is nevertheless given.

Anoestrum: It is believed to be normal for wild cattle to have a definite breeding season. The calves are born in the spring or early summer (Hammond 1927). This would mean that any female which does not become pregnant within the first 3 to 5 months following calving becomes anoestrous during the late autumn and continues to be so during the winter and early spring. She will not shew signs of "heat" until late the following spring. The conditions of improved nutrition under which the vast majority of our domestic cattle are kept have apparently enabled them to dispense with a breeding season as such and to "come on heat" at all times of the year when they are not in calf. Under conditions of stress there is a tendency for them to revert to the primitive and to fail to "come on heat" during the period when this stress applies. The extreme reproductive reaction to stress is anoestrum.

As one might expect, anoestrum is most commonly displayed during the winter months. The usual subjects are heifers on a low plane of nutrition and cows milking heavily and on a diet which will barely provide for body maintenance and the production of the amount of milk being secreted. As might also be expected, it is easier to prevent anoestrum than to cure it once it has become established. The methods of prevention are fairly obvious.

Attempts to cure the condition by the use of oestrogens and by the administration of follicle stimulating hormone (F.S.H.) in the form of pregnant mare serum meet with comparatively little success. Nor is it surprising that this should be so. To administer F.S.H. and expect to get maturation of follicles accompanied by oestrum, ovulation, luteinisation, and growth and maintenance of the corpus luteum is like "whipping a tired horse" and expecting to get the degree of response one would elicit from a "fresh animal".

Oestrogens will sometimes produce "symptomatic heat" but such heat is generally not accompanied by the initiation of any reproductive cycle (nor, for that matter, of any hormonal cycle) in the ovary. Therefore, if mating does occur, it is almost certain to be fruitless.

During the early years of the last war extravagant claims were made for the efficacy of pregnant mare serum in the treatment of anoestrum. Extensive field trials by the present author and by other workers shewed that p.m.s. alone or in combination with stilboestrol dipropionate, was of very little if any use in the practical treatment of heifers and cows exhibiting true anoestrum. Clinically appreciable reaction in the ovary often did occur in the form of folliculation (frequently multiple) but only seldom did ovulation and signs of oestrum accompany this folliculation.

Where true anoestrum is demonstrated during the winter little hope can be offered for any improvement before about mid-April. During the early spring (from the latter half of March onwards) if cattle are in reasonable bodily condition a small dose of stilboestrol dipropionate (20-27·5 mg.) may be given intra-muscularly in the hope that it may "trigger off" the hormonal and reproductive cycles in an ovary which would in any case soon begin to function normally if no interference were made. Alternatively, p.m.s. (1500-3000 units F.S.H.) may be given subcutaneously, intramuscularly, or intravenously with the same object. It should be noted that when freeze dried p.m.s. is used intravenously the danger of anaphylactoid reaction exists if animals have previously been sensitised to horse serum. The author has encountered four such cases. In two of these the reaction was slight but in the other two there was immediate collapse of the animal and extreme distress. All four recovered spontaneously.

Suboestrum: Cases of suboestrum are commonly encountered particularly among dairy cows, and among cows which are being used for the multiple suckling of calves. Two lines of treatment are available, viz.: (a) manual expulsion of the corpus luteum and (b) the administration of oestrogen by intramuscular injection.

Removal of the corpus luteum by manual expulsion is usually easily accomplished if attempted during the period of its greatest bulk. In order to achieve this it may be necessary to examine the patient twice, allowing about a fortnight to elapse between the first and the second examinations. When the corpus luteum is expelled oestrum may be expected to follow in from 2 to 10 days (usually 4 or 5 days). Expulsion should not require the exertion of much physical pressure and if such seems to be necessary the operation should not be proceeded with. The operation is not entirely without risk to the animal. Severe haemorrhage may occur. The author has experienced the death of two animals from this cause following manual expulsion. He does not recommend the procedure for that reason and seldom now employs it. When it is undertaken it is recommended that the thumb should be inserted into the fossa from which the corpus luteum has been expelled and slight pressure applied with a view to controlling bleeding.

The administration of oestrogen seems to be generally sounder practice. According to the size of the patient 20-27·5 mg. of stilboestrol dipropionate may be given intramuscularly. Symptoms of "heat" may be expected in from 2 to 10 days. Usually the interval between injection of oestrogen and the ensuing "heat" is about 4 or 5 days. Large doses of oestrogen should not be used because of the danger which exists of their seriously interfering with the ovarian cycles, hormonal and reproductive. Hancock (1947) has already drawn attention to this danger.

After response to treatment, as measured by the occurrence of oestrum, cases of suboestrum may relapse or may even become anoestrous. It is, for that reason, advisable that an early examination for pregnancy should be carried out on all cases which have been apparently successfully treated.

Cystic Disease of the Ovaries: In contrast to the last-mentioned condition, cystic disease of the ovaries presents a very real problem to the clinician. The degree of success which is met with in treatment varies enormously in different cases. The reason for this great variability in response to treatment probably lies to some extent in the fact that "cystic disease" is not an etiological entity but rather a group of conditions all of which are characterised by the appearance in the ovaries of abnormal bodies which are morphologically described as "cysts". Histological examination of these bodies shows that they may be follicular cysts or luteal cysts. There are also cystic corpora lutea. These last may be eliminated from this discussion since they appear to behave in roughly the same manner as normal corpora lutea.

It seems logical to suppose that different forms of therapy should ideally be applied depending on whether cysts are follicular or luteal. Or even, if the nature of the therapy should not be radically different, one might suppose that the scale of dosage of hormones required to correct the different abnormalities would vary considerably. Nevertheless, owing to the difficulty, or perhaps the impossibility, of differentiation in practice, we tend to treat all cases of cystic disease indiscriminately by a routine therapy. It is little wonder that we find it difficult to make a reliable prognosis in so many cases.

It is the author's practice to attempt manual rupture of all cysts on the occasion of the first visit to a case and to make a second visit about 8-10 days later, or when next the patient shows signs of "heat", whichever is the earlier. If signs of "heat" occur first, the animal is re-examined and, provided no cyst is detectable, 1500 i.u. of luteinising hormone (L.H.) are injected intravenously. The case is then revisited after a further period of 8-10 days to determine whether a corpus luteum has formed. If a corpus luteum has formed, 1500 i.u. of L.H. are given intramuscularly with the object of assisting in its maintenance. It is realised that any benefit which may be derived from this second injection of L.H. is possibly not attributable to the L.H. itself but rather to impurities of luteotrophic hormone (L.T.H.) present in the preparation. If, when this third visit is made, it is found that what appears to have been luteinisation without ovulation has occurred, the luteal tissue is manually expressed or a dose of 20-25 mg. of stilboestral dipropionate is given by intramuscular injection. At the ensuing "heat" 3000 i.u. of L.H. are given intravenously, and then a further 1500-3000 i.u. of L.H. ten days later, if a corpus luteum has formed.

If, on the occasion of the second visit (i.e., the visit 8-10 days after the initial rupture), another cyst has formed this is ruptured manually and a dose of 1500 units of L.H. given intravenously at the same time. The case is revisited about 8-10 days later and treatment proceeds as has been described.

Using the routine outlined above the author believes that he obtains the best results. It will be noted that when L.H. is used with the object of inducing ovulation and luteinisation it is administered by the intravenous route. It is believed that by using this route better results are likely to be obtained than when the intramuscular route is employed. On the other hand, when L.H. is administered with the object of maintaining the corpus luteum (possibly as a result of L.T.H. impurity in the preparation) the intramuscular route is chosen. It is felt that for this latter purpose a slow and more prolonged action would seem to be desirable. Ideally for this purpose an implant, or an injection, of slowly absorbable L.T.H. would be preferable.

Hancock (1947) found that out of 12 cows and heifers with "cystic ovaries" which were given 800-3,200 i.u. of L.H. intravenously 7 became pregnant after a total of eleven services. Hinze (1959) reports that of 113 cows with "cystic ovaries" 67·5 per cent. conceived after treatment with differing preparations containing L.H. He believes that the best results are obtained when the cysts are ruptured or drained at the time of administration of L.H. Dawson (1957) quotes various authors who produce evidence to suggest that hormonal treatment gives better results than rupture. On the other hand, one worker quoted by Dawson, viz., Danelius, comes down very heavily on the side of rupture as opposed to hormonal treatment. It should be noted however, that Danelius apparently used very small doses of hormone and em-

ployed the intramuscular route. These factors may explain the poor results he obtained from hormonal therapy.

As far as mechanical rupture is concerned, Cunkelman (1948) reports that in 60 cases so treated 36·6 per cent. subsequently shewed a normal ovarian cycle and conceived. Dawson's own work (1957) suggests that manual rupture of cysts on one or more occasions at 10-day intervals might, as judged by *post-mortem* examination, be expected to give results at least as good as those obtained by Cunkelman and possibly somewhat better. The present author would consider results of that order obtained as a result of rupture alone to be about what he might expect. The general consensus of opinion seems to be that, while both rupture alone and hormone alone can give reasonable results, the latter treatment, particularly when the intravenous route is chosen, is the better. Best of all is perhaps the combination of manual rupture and hormone therapy.

In choosing as between manual rupture and hormone therapy it is interesting to note that Hinze (1959) records a correlation between twinning-rate and the mode of treatment employed in the treatment of ovarian cysts. He found that cows with unexpressed cysts (presumably treated by hormone therapy) had a twinning-rate of 4·3 per cent. which was essentially the herd average. Cows whose cysts had been manually ruptured had a twinning rate of 11·3 per cent. There was also a negative correlation between the twinning-rate and the length of time between rupture of the cysts and conception. Those conceiving within 25 days after rupture had a twinning-rate of 23·3 per cent., those conceiving between 26 and 75 days after rupture 15·7 per cent., and those conceiving after 75 days had the same twinning-rate as the rest of the herd. (Hinze also observed that of all the cows which displayed signs of "heat" during pregnancy 71·3 per cent. had suffered from cystic ovaries before becoming pregnant.)

Some veterinarians have used progesterone in the treatment of cysts and claim good results from this form of therapy. The author has had little experience of its use for this purpose. Hinze in the paper quoted above says "other hormones, such as progesterone, have had little value in the treatment of cystic ovaries in cows in this herd".

Dodson (1958) discussing the use of hormones in the treatment of certain types of infertility concludes that improvement in the results of hormone therapy will occur only when experience in the diagnosis of types of infertility in relation to the endocrinological picture is more advanced; when, in other words, it is possible to select the correct hormones on a basis of clinical examination. Nowhere in the field of bovine gynaecology is this more true than in the treatment of "cystic disease" of the ovaries.

Research workers have shewn that follicular cysts, luteal cysts, and cystic corpora lutea exist. Follicular cysts, if not luteal cysts, have been further classified according to whether they are active or apparently inert endocrinologically. If Smythe, Dawson, and Hancock are correct in their belief that cystic corpora lutea tend to prolong the ovarian cycles we may assume that cystic corpora lutea are endocrinologically active. In any case, corpora lutea, cystic or normal, tend to be comparatively easy to differentiate from follicular, but possibly to a lesser extent from luteal, cysts so that they should not normally present a problem to the general practitioner. But how is the general practitioner to distinguish between follicular cysts, active or inert, and luteal cysts? The ability to differentiate clinically between these forms must eventually have a bearing not only on therapy but also on prognosis.

In the discussion on technique the suggestion was made that the thickness of the wall of the cyst, i.e., the ease or otherwise with which it could be ruptured, might be taken as a guide as to whether a cyst was probably luteal or otherwise. The history of the case may also give some indication. Dawson (1957) says that luteal cysts appear to be characteristic of virilism. If that is so, may one conclude that, in a cow which shews signs of virilism, cysts in the ovaries are probably luteal? Similarly, the cysts in a nymphomaniac cow would seem most likely to be follicular and active. Where the animal shews no sign of heat nor any sign of virilism the only guide one has as to the type of cyst present is the thickness of the wall, if indeed that is any real guide.

Unless cystic disease has been of short duration a very guarded prognosis must, in the present state of knowledge, be given. If cysts appear to be follicular and not associated with nymphomania the author tends to give a more hopeful prognosis than when nymphomania is present and a considerably more hopeful prognosis than if the cysts appear to be luteal and associated with virilism. In the last event the prognosis is bad.

Delayed Ovulation: Delayed ovulation is known to occur sometimes. The author has observed cases in which he is certain, so far as one can be by rectal examination, that it has occurred. These cases have been few. More often, in the inability to demonstrate any cause for infertility, he has suspected delayed ovulation and suggested it as a possible cause.

In view of the fact that manganese deficiency has been suspected as a cause of delayed ovulation in some species he prescribed manganese to be given orally.

He pictures delayed ovulation as possibly a stage on the road to the development of follicular cysts. For that reason he administers 1500-3000 units of L.H. intravenously while the animal is "on heat". He generally examines the animal again in about 8-10 days and, if the corpus luteum appears to be poorly developed, gives 1500 units of L.H. intramuscularly. Following this treatment many animals become pregnant and fewer, so the author believes, do not.

Hinze (1959) recognised the condition. His line of treatment differed radically from that already suggested. He used F.S.H. intramuscularly 2 or 3 days before the next anticipated oestrum or F.S.H. intravenously early in oestrum. In one trial involving 58 cows, only 12 conceived. The idea of using F.S.H. in the treatment of this condition (especially of using

it intravenously during oestrum) seems novel. One might imagine that any beneficial effects obtained would be the result of L.H. impurity. Perhaps, on the other hand, the condition which Hinze describes as "delayed ovulation" is different from what is understood by that term among British veterinarians. Might it be more correctly described as "subfolliculation"?

Amoroso (1955) writing on the hormonal control of the oestrous cycle said, "We know, as did the ancients, that breeding seasons occur, but as yet we are still ignorant of the precise combination of factors that initiates the sexual rhythms of even a single vertebrate. The nearest we can approach the latter is to say that the seasonal maturation of the sex organs of various species must be viewed as a mosaic of events, the patterning of which depends on an intimate and coalescent interaction of intrinsic and extrinsic factors".

When undertaking the treatment of infertility in the cow, particularly of infertility which we believe to be functional, it is well to reflect on Professor Amoroso's remarks. All we may truthfully say is "now we see through a glass darkly". Perhaps, in the course of time, our successors will see "face to face".

References

AMOROSO, E. C. (1955). *Vet Rec.*, **67,** 1072.
CRAIG, J. F. (1937). *Ibid.*, **47,** 479.
CUNKELMAN, J. W. (1948). *J.A.V.M.A.*, **112,** 292.
DAWSON, F. L. M. (1956). *Proc. III Int. Cong. Anim. Rep.*, **2,** 46.
——. (1958). *B.V.J.*, **114,** 94.
——. (1958). *Ibid.*, **114,** 134.
——. (1958). *Vet. Rec.*, **70,** 487.
——. (1957). *B.V.J.*, **113,** 112.
——. (1959). *Ibid.*, **115,** 46.
DEMETER, H. (1926). *Münch. Tierarztl. Wschr. Jahrbg.*, **77,** 514.
DODSON, M. E. (1958). *Aust. Vet. J.*, **34,** 291.
DONALD, H. P. (1943). *Vet. Rec.*, **55,** 297.
DYKSTRA, R. R. (1955). *Vet. Med.*, **50,** 224.
GOULD, G. N. (1942). *Proc. Roy. Soc. Med.*, **35,** 635.
——, HIGNETT, S. L., and STEELE-BODGER, H. W. (1942). *Vet. Rec.*, **54,** 69.
HAMMOND, J. (1927). *Reproduction in the Cow*. Camb. Univ. Press.
HANCOCK, J. L. (1948). *Vet. Rec.*, **60,** 513.
——. (1947). *Ibid.*, **59,** 639.
HANLEY, S. (1953). *Irish Vet. J.*, **7,** 142.
HIGNETT, S. L., and HIGNETT, P. G. (1953). *Vet. Rec.*, **65,** 21.
HINZE, P. M., (1959), *J.A.V.M.A.*, **134,** 302.
JOB (ca. B.C. 1520). *The Book of Job*, ch. 21, v. 10.
LINDLEY, D. C. (1954). *J.A.V.M.A.*, **124,** 187.
MOSES (ca. B.C. 1491-ca. B.C. 1451). *Genesis*, ch. 31, v. 38.
MURRAY, J. G. (1943). *Vet. Rec.*, **55,** 323.
——. (1939). *J. Comp. Path.*, **52,** 135.
——. (1942). *Vet. Rec.*, **54,** 514.
ROWSON, L. E. A. (1942). *Ibid.*, **54,** 74.
SMYTHE, R. H. (1943). *Ibid.*, **55,** 16.
SPRIGGS, D. N. (1945). *Ibid.*, **57,** 469.
STUHLENMILLER, M. (1933). *Tierarztl. Rdschr.*, **39,** 377.
TRAUTMAN, E. C. (1954). *J.A.V.M.A.*, **124,** 184.
VIGUE, R. F., FITZGERALD, W. H., and CASTRUCCI, R. F. (1959). *J.A.V.M.A.*, **134,** 308.
WILLIAMS, W. L. (1921). *The Diseases of the Genital Organs of Domestic Animals*. Andrus and Church, N.Y.
WILLIAMS, W. W. (1924). *Amer. Vet. J.*, **65,** 695.
WRIGHT, J. G. (1945). *Vet. Rec.*, **57,** 313.

THE SPEAKER'S INTRODUCTION

The Chairman, Mr. G. S. Peyton, introduced the author of the paper, and asked him to present the paper.

Mr. J. G. Murray, in presenting his paper, thanked the Association for the honour it had done him in inviting him to give the paper to the Congress. When he had accepted the invitation, which he had done with great trepidation, he had realised that there was little he could say on the subject of infertility in the cow. When actually writing the paper, he had realised that there was nothing that had not already been said by others. He had also realised that a great deal of what had been said (by himself as well as by others) regarding infertility in the individual cow had been unsupported by the evidence of experiment and control. That this had been so had been inevitable, since much of what had been written had been written by those who were engaged in practice, and the keeping of accurate records and adequate controls was, to say the least, certainly extremely difficult in the rush and turmoil of general practice.

Often success had been claimed for a line of treatment on the most slender of evidence. One tended to forget that the majority of cases presented for treament were temporarily rather than permanently infertile, and that these would tend to recover spontaneously provided that the treatment that was administered was not definitely harmful. The experiment that had been carried out by Dodson in Australia might be cited to illustrate that point. Dodson, when attempting to find out the efficacy of a certain line of treatment on a series of sterile cows, had treated 38 and left 100 as untreated controls. He had found that 57 per cent. of those untreated controls had become pregnant within 125 days of the 85th day post partum. That meant that if infertile cattle were presented for treatment soon after the 85th day post partum, one might expect that over half of them would eventually become pregnant even if no treatment at all were applied. Dodson had suggested that the only true criterion of the efficacy of any treatment was the amount by which the lag phase (the period between the 85th day post partum and the day on which conception occurred) was shortened. How many of the recommended treatments could stand up to that criterion?

He had attempted to present the evidence for and against some lines of treatment which might be applied to cases of certain types of infertility. Some of those present might have found time to read through the paper. The majority, he was certain, would have found more pleasant ways of passing the time in Folkestone. Nevertheless, he did not intend to read out a paraphrase of what he had written. In the first place, to do so would be extremely boring, and in the second place, it would encroach upon the time allotted to the opener and to the general discussion. On a subject such as this, open discussion was likely to prove more interesting than the formal reading of an address.

There were, however, one or two points to which he wished to make reference.

It was the custom for many to classify a case of infertility as "of functional origin" when they could find no apparent abnormality in the tubular portion of the reproductive tract (i.e., the uterus, vagina, etc.) and its appendages. It would be interesting to know what proportion of those cases was associated with undetected changes in the fallopian tubes resulting in those organs becoming impassable to the ovum.

In this connection, the excellent work of Hanley in adopting Rubin's tubal patency test for use in the cow should be noted. Though it would appear that this method of tubal insufflation was likely to prove too risky for use in general practice, perhaps some of the specialist workers had had experience of its use and could give some information regarding their findings by this method of examining the salpinges.

In his paper he had referred to a test described by McDonald in America. This test, though perhaps likely to be less exact than the Hanley-Rubins test, would seem to be more applicable to general practice. Had any work been done in this country along those lines?

In the meantime, he would remind them of the Nielsen-Williams technique for manual palpation of the ovarian bursae and fallopian tubes. He was constantly being surprised by the number of younger veterinary surgeons who did not use this technique as a routine in the examination of cases. It was not a difficult technique to master, and it had been clearly shown by Dawson that a very high degree of accuracy could be obtained in detecting macroscopic lesions in the bursae and salpinges.

Many veterinary surgeons seemed to regard the diagnosis of pregnancy of less than about 7 weeks' duration as some-

thing which could not be undertaken by anyone other than a specialist. It was his own experience that at no stage was pregnancy so easy to diagnose as it was at 5 to 5½ weeks—except after the sixth month. Often a positive diagnosis could be made at about 30-32 days. It was unwise, however, to make a negative diagnosis at less than 5 weeks unless the findings in the ovary precluded the possibility of pregnancy.

It had been argued that, since the limits of variation of the diameter of a non-gravid uterine horn overlap with those of a 6 weeks gravid horn, it was not possible to state accurately whether pregnancy of less than about 7 weeks was present. That contention was not valid since the interpretation of the findings on rectal palpation were made on a qualitative as well as on a quantitative basis. The "feel" of the organ was even more important, within limits, than the size. In addition, one had the non-gravid horn for comparison.

The significance of vaginitis as a factor in bovine infertility had been the subject of much controversy. He had always believed that vaginitis was not a common cause of infertility. He had frequently seen what had been described as granular vaginitis in herds which had no history of infertility. Nevertheless, he wished to draw attention to the findings of Trautman. Trautman reported the finding of granular vaginitis in 51 per cent. of some 4,600 cows at the time of insemination. Cows showing mild symptoms had a fertility rate 3.2 per cent. lower than non-affected cows, and cows showing severe symptoms 10.8 per cent. lower than had non-affected cows. Trautman's experience suggested that vaginitis might be a cause of infertility. Diagnosis had been made by lay inseminators, and it was not clear how many of those cases were cases of vaginitis *per se*.

In regard to edometritis and pyometra, he wished to stress the prime importance of attention to any ovarian abnormalities which might be present. In the case of pyometra he would suggest that one of the most frequent predisposing causes might be over-oestrogenisation of the uterus. Such over-oestrogenisation might possibly have taken place even though the animal had never shown symptoms of oestrum.

On the question of cystic disease of the ovaries, in considering the treatment of cystic disease cases Hinze's findings were of interest with regard to the increased twinning rate he had observed among cows where cysts had been ruptured manually. It would also be noted that th. increase in twinning rate was inversely related to the length of time between rupture of the cysts and conception. .

Hinze's evidence suggested that the increased twinning rate was associated with the exertion of a degree of pressure on the ovary. If this was in fact the explanation, might one expect to observe a similar increase in twinning rate following manual expulsion of the corpus luteum?

The Opener

Mr. J. A. Pasfield, who opened the discussion, said that in reading the published works of veterinary obstetrical authorities one was impressed by the tremendous weight of facts and figures that were produced. They had indeed slain their tens of thousands. The subject as it concerned the manipulation of the individual had also been described in the most careful detail. This was the well-known reason why the practitioner of to-day stood at a disadvantage in the collection of vast masses of data, since other pressing activities, sometimes not unconnected with the State, engaged much of his attention, and, as the meeting knew, the amount of general writing which went on in any practice nowadays would have been too terrible to contemplate by their predecessors. But the list of references in Mr. Murray's paper was impressive. It showed a wide range of reading over a substantial time, and this, under the conditions of general practice, was no mean achievement. His references ranged from writers of the Old Testament to authorities of to-day. It was a matter of pride to note how many of the modern references were supplied by Great Britain, but a little disconcerting to see that some of their authors were present that day!

In few veterinary fields did the individuality of the examiner emerge so clearly as in the diagnosis and treatment of individual bovine infertility. Whole schools had arisen in opposition to each other. Diagnosis was often tempered by private opinion, by experience and even by force of character. But one could not fail to observe a certain similarity of viewpoint between the late Professor Fölmer Nielsen and St. Paul (who, he thought, might have had an acknowledgement for Mr. Murray's quotation from the First Epistle to the Corinthians), since in an earlier chapter he wrote "And if any man thinketh that he knoweth anything, he knoweth nothing. . . ." This was a safe point at which to start thinking. Though they well realised that Nielsen's monumental clinical examinations laid the foundations upon which the old Survey Committee so brilliantly built a working system for the practitioner, his clinical records presented to the Congress of the Association in Dublin in 1926 were remarkable for the humility of their judgements and abounded in such notes as "? normal", "? endometritis", "? lutein cyst", "? corpus luteum". If an acknowledged master could so far refrain from dogmatism it behoved us then to be humble about their opinions. But "Observation", said Meredith, "is the most enduring of pleasures of life", and that was Mr. Pasfield's excuse for speaking.

It was doubtful if such a thing as a "national" picture of infertility existed in the individual animal. The condition even varied from county to county. It was certainly influenced by geographical and climatic factors, and even more so by the methods of animal husbandry practised from farm to farm, by the class of stock under consideration and by the methods of milk production. Mr. Murray thought a national picture of suboestrus and anoestrus existed among cows and heifers during and immediately after the second world war. It seemed to Mr. Pasfield at that time that this was a man-made sequel to the drive for winter milk, coupled with the generally poor treatment accorded at that time to the non-productive female, and that the condition became accentuated in the next one or two generations of young females which had been forced into an unready world at an unnatural time.

Mr. Murray had rightly laid stress on the dangers in practice of assuming that recent oestrus, or conversely no evidence of mating, indicated non-pregnancy. These hazards become very much more important with the widespread use of artificial insemination and the reliance placed on the owner's powers of observation. Privately he held the opinion that an early pregnancy diagnosis service was inseparable from the successful practice of artificial insemination, but he did not propose to lay himself open to slaughter by suggesting how it should be instituted. In the matter of early pregnancy diagnosis, Mr. Murray had said that it was easier to be accurate at 5 to 5½ weeks than at 7. He did not wish to dispute this point, particularly in so far as it concerned maiden heifers, but the technique involved not only the palpation and assessment of the uterine state but the manipulation of one or both ovaries as an adjunct. Undoubtedly the method was theoretically superior to that of palpation alone, but only in the hands of a competent operator. In this class of work the road to competency was hard, long and dirty. The appreciable incidence of early foetal absorption could present a problem to the practitioner who had declared an early pregnancy to exist, only to be confronted later by normal oestrus. The fate of the early embryo in many herds was sufficiently precarious to warrant a fortnight's delay until the passage of the second post-service period, when great reliance could be placed upon the feel and texture of the gravid horn to which Mr. Murray had referred. For the same reason he was wary of the technique which involved picking up the foetal membrane and allowing it to fall away from the endometrium.

Mr. Pasfield felt that (within the limits of an epidiascope) the best way to comment upon this excellent and comprehensive paper would be to take out an abstract of last year's examinations for pregnancy and infertility in practice and to see whether they accorded with the degree of importance given to them in Mr. Murray's estimation. Naturally there were severe limitations about such a method: the analysis was compiled from working day-sheets and might therefore be thought to be rather rough and ready. The headings were excessively brief and made no allowances for such details as, for instance, the character or bacteriology of uterine discharges, such doubtful entities as vaginitis, nor (in the instance of

retained placenta) whether the parturition was at full term or premature. The records did not represent the work of the whole practice but only of one member. In this case they referred only to purebred cattle, so the age incidence was generally fairly high, and sometimes treatment had been persevered with or time spent upon beasts which, under other circumstances, might be thought to be uneconomic. On the other hand, this small survey gave a broad general picture of seasonal changes which confronted the general rather than the specialist gynaecologist.

He had begun by listing the number of routine pregnancy examinations carried out monthly in order to give a proportionate picture between the normal and the abnormal cases encountered in practice.

It would be seen that the states of suboestrus and anoestrus presented no problem until December when, as one might expect, a rise to 93 was noticed in the following 3 months after only 28 cases had been recorded in the preceding quarter. But the climatic conditions of the autumn of 1958 in West Sussex, as he believed of the British Isles in general favoured the continuous growth of grass until well into the winter. This herbage proved of no value in the maintenance of cycling oestrus and was in fact deceptive, since stockowners placed too much reliance on it and would have been better advised to begin supplementing this grazing from August onwards with some concentrate or hay.

The schedule indicated that twice as many cases of suboestrus (according to the definition given by Mr. Murray) were treated by the expression of the corpus luteum than by the use of oestrogen, luteinising hormone or P.M.S., and that luteinising hormone was more widely used than follicle-stimulating hormone. In the smooth anoestrus heifer in particular, it was found that 1,500 to 2,000 units of L.H. intramuscularly was frequently followed by the production of a good quality corpus luteum which, when examined at the tenth day, might be expressed if the operator thought it desirable, or might be allowed to remain undisturbed while attention was being paid to the general physical level of the animal. If this might be thought illogical he could only say that it worked well in the featureless ovary and one

			1958 April	May	June	July	August	September	October	November	December	1959 January	February	March
NORMAL PREGNANCY		COW	51	46	40	44	16	51	61	34	60	63	54	88
		HEIFER	31	76	19	43	14	63	49	19	42	52	65	70
APPARENTLY NORMAL			3	—	1	1	2	1	2	2	1	1	3	2
INFERTILITY	METRITIS	NO DISCHARGE	6	12	2	13	1	5	11	8	19	15	12	20
		ACTIVE DISCHARGE	17	26	20	12	3	14	20	21	37	22	18	22
		SALP: & BURSITIS	3	1	2	2	—	1	—	—	—	1	—	1
		CERVICITIS	1	2	3	2	—	3	—	—	2	6	2	2
		CYSTIC OVARY	1	3	1	1	—	—	1	2	5	7	10	5
	ABORTION	TWIN	1	—	—	1	—	—	—	—	—	—	—	—
		SINGLE	2	1	6	3	4	5	5	3	2	3	7	2
	RETAINED PLACENTA	TWIN	5	—	1	2	—	3	2	—	2	1	—	1
		SINGLE	4	3	7	5	4	11	4	4	9	7	7	4
	MUMMIFIED FOETUS	COW	1	—	1	—	—	—	—	1	—	—	—	—
		HEIFER	2	2	—	1	—	1	1	—	—	1	—	—
	VAGINITIS	COW	—	—	2	1	—	—	—	—	—	—	—	—
		HEIFER	2	—	—	—	—	—	—	—	—	—	—	—
	ANOESTRUS	EXPRESS COW	2	6	3	8	—	4	6	7	16	16	14	15
		EXPRESS HEIFER	9	4	7	3	—	1	1	1	4	8	6	2
		LUTEINISE	2	5	6	3	—	2	1	5	4	11	12	9
		STILBOESTROL	2	3	3	—	—	—	—	—	—	—	2	1
		P.M.S.	1	—	1	3	1	—	—	—	—	—	—	—
	L.H. AFTER SERVICE		1	3	4	5	3	5	4	4	3	9	6	3
	PROGESTERONE IMPLANT		—	—	—	1	1	2	1	2	5	1	—	1
TOTAL INFERTILITY CASES			62	71	69	66	17	57	57	58	108	108	96	88
PERCENTAGE OF ACTIVE METRITIS IN THE TOTAL			27·4	36·6	21	18·2	17·6	24·6	35	36·2	34·3	20·4	21·4	25

TABLE 1

could keep as a second line of attack the FSH with its possible hazard of superovulation.

He was well aware of the serious differences of opinion which existed about the advisability or otherwise of inducing oestrus by the manual expression of the corpus luteum. Undoubtedly the method had disadvantages and even dangers in the hands of the over-enthusiastic or indiscriminating. Markowitz, in his masterly introduction to "Experimental Survery", quoted the late C. H. Mayo as saying that some people may make the same mistake a hundred times and still call it experience. This might be so of enucleation. But it seemed reasonable to say that if the corpus luteum was well defined and mature, well raised above the surrounding parenchyma, clearly grooved at its periphery, easy to enucleate, then—in the absence of any other genital lesion (a rather important proviso)—it could be enucleated with a low degree of risk. But he thoroughly agreed with Mr. Murray that on no account should effort be made to manipulate immature, resistant or ill-defined corpora lutea, particularly were suboestrus was the sequel to uterine infection with *C. pyogenes* and where the ovary was shrunken and dehydrated to the touch.

The meeting might have observed that the course of a year's work brought 45 observable abortions—far too high a figure. Of these only 2 were attributable to *Br. abortus*, both from a herd in which S.19 vaccination had been practised for years. Of more interest was the incidence of fungal abortion beginning in October 1958. Seven cases in 4 herds had occurred by the end of this resumé; from the first 3 the fungus was not typed, but of the remainder, 3 were attributed to *Aspergillus fumigatus* and 1 to a Mucor-type fungus. For these investigations he was greatly indebted to Mr. Austwick at Weybridge. Of the 7 cows, 2 were slaughtered as uneconomic, the remaining 5 being irrigated after uterine involution with domiphen bromide solution aqueous 1/5000, and 4 were again pregnant. It remained to be seen whether the use of a specifically antifungal irrigant was superior to that of a non-specific solution.

It was obviously important that the examination of the genital tract should be correlated to that of the whole animal. The cow suffering from active mastitis was unlikely to conceive, and such conditions as actinobacillosis or necrobacillosis of the hoof, would militate most severely against conception. For example (looking at this picture the other way round), in assessing the value of an indefinite complement fixation test in the diagnosis of Johne's disease he would be inclined to go rather carefully into the breeding history of the animal, since experience showed that persistent anoestrus in the heifer and repeated foetal absorption were early signs of infection, but simple examination of the internal genitalia would produce no reason for the infertile state.

He had included a heading for cervicitis, and he believed that he had found 23 cases during the year. He could not help thinking that the condition occurred more frequently than was generally thought, and he was filled with admiration at the grimness and persistency of the bovine uterine cervix in struggling on against overwhelming odds. This unfortunate and much-abused organ even in its natural state offered such a variety of shapes and sizes and textures that one needed to be careful before saying that it was unable to fulfil its many functions. As criteria of abnormality he would suggest that the hypertrophic cervix which disproportionately occupied the pelvic basin after involution, or one through which the careful passage of the uterine catheter was very difficult or impossible, or one in which it was possible to palpate large fibrotic masses, qualified for diagnosis as cervicitis; is was almost certainly part of a general endometritis, whether palpable or not, and should probably be treated with a lugol irrigation which would infiltrate through the cervical canal at a slow rate. In all cases Mr. Pasfield left a communication for the inseminator (where this was practised) asking him to be most careful about his intra-uterine technique and to abandon the attempt if it proved difficult. In this connection he would like to say that in order to achieve better results in the treatment of individual infertility he felt that it was in the interest of the practitioner, and certainly of the stockowner, that excellent relations should exist between veterinary surgeon and inseminator on the basis of mutual respect.

Mr. Murray did not consider cervicitis to be an important cause of sterility and quoted Wright as of the same opinion. Fleming recognised stenotic cervicitis as an entity worth discussing, but Hammond quoted frequent cases of cows and heifers in regular oestrus and in which the organs appeared anatomatically normal where he considered the infertility to be due to cervical abnormality. If one's routine questions included the sex of the preceding calf one might be surprised at the frequency with which the answer was that of a bull. The Opener drew attention to the columns headed "Endometritis", and to the disproportionate number of metrites over all other causes of individual infertility. Of 857 individual cases examined, metritis was considered to be the causal factor in 390 cases (or approximately 45 per cent). He believed that the greatest single reason for individual infertility was trauma, whether observable or not, and that the reason for the high incidence of secondary infection of the uterus lay with domestication and the phenomena which Dr. Taylor had so well described on Tuesday. There was nothing novel about this view: Mr. Pasfield believed it to be held by the majority of practising veterinary surgeons. But, if it be accepted that primary trauma was a more widespread condition than was generally accepted, such an injury might be self-inflicted in labour and might not always be the work of man, and the logical move was to remember this when considering the production of crossbred beef stock and to remember that the production of calves having excessive trans-cranial or (more important) trans-pelvic measurements was likely, under intensive conditions, to produce not a new problem but an exacerbation of an old one with which they had been struggling for many years.

The General Discussion

Dr. F. M. Dawson (Cambridge): "Mr. Murray has observed a very high ethical standard in disclaiming the use of his name for a cow gynaecological technique devised by Nielsen, especially as the catheter designed by Albrechtsen is always known in this country as a Nielsen catheter!

"The recent abattoir surveys of Holcombe in Finland, Bainer in Germany and Fujimoto in Japan provide evidence in favour of Roberts (1955) conclusion that the incidence of cystic ovarian disease is rising rapidly, despite its low incidence in Mr. Murray's practice.

"In answer to Mr. Murray's queries, the tubal patency insufflation test is difficult to apply in the conditions of practice, and in any case it appears likely that 35 per cent. of tubes impassable to ova are permeable to fluid. Mr. Donald's test using starch grains intraperitoneally injected is more promising. A series of service intervals within the normal range of 18-25 days are a useful indirect indication of tubal closure, and *vice versa*."

Mr. S. Jennings (Glasgow): "It is difficult to compare the reports of different workers regarding cystic ovaries in cattle. Williams some 30 years ago and more recently Garm, Roberts and others appear to have encountered large numbers of cases but they failed to state how long the animals had been affected before the diagnosis was made. It is not possible always to make a diagnosis after one rectal examination but only after a series of examinations. It is difficult to know where delayed ovulation finishes and cystic ovarian degeneration begins.

"Mr. Murray has very rightly referred to the tendency to spontaneous recovery in cases of infertility. I find this particularly true of cystic ovarian degeneration. I purchase clinical cases in order to keep them under observation for at least 6 weeks. The animals which are available for purchase have almost always been affected for some time, but in spite of being chronic cases they often make spontaneous recovery during the 6 weeks' observation period."

Mr. G. F. Smith (Thames Ditton): "Firstly, Mr. Chairman, may I congratulate both Mr. Murray and our opener on the excellent way that they have dealt with an extremely complex problem.

"I would like to refer to Mr. Brown's comments on the incidence of abortion following artificial insemination. The whole industry has looked at this question quite seriously and we believe that it would be wrong for us in any way to change our training practices in order to avoid the extremely small percentage of such accidents that do arise.

"Turning to the whole field of reproductive failure, I think we must first and foremost understand that the

economic value of the female is on many occasions the point which is considered by the owner. At the present time the salvage value of the dairy cow in many breeds does not warrant a long course of treatment and a considerable dry period. Therefore, it is probably from this standpoint that we have to approach the whole of the fertility problem.

"On the part of the A.I. movement at least, many of us feel that our biggest problem is management and sub-fertility; the detection of heat and the presentation of animals at the right period in the cycle are, no doubt, factors which call for a high degree of stockmanship. On many occasions this is not forthcoming, with the result that an unnecessary number of returns to service result.

"Foetal degeneration has been mentioned as a possible cause of wastage and I think this has been substantiated by Dr. Laing and such workers as Casside and Tenarby in America. It is, in fact, surely an accepted understanding that there must be a certain degree of foetal loss during the first few days or weeks of pregnancy and, therefore, it may be difficult for us to improve to any marked degree our percentage of return to first service.

"The author of the paper has admitted to the lack of knowledge in the treatment of infertility. Very little has been done to provide control groups in this field and it is quite obvious that more detailed work ought to be done before any assessment of the value of different treatments can be made. It is unfortunate that we have little available information on the outcome of treatments from Scandinavian countries and Canada where the A.I. organisation includes these additional services."

The Reply

Mr. J. G. Murray, in replying to the discussion, thanked Dr. Dawson for his information with regard to the Hanley-Rubins test and the McDonald test. He was sorry if he had given the impression in the paper that he regarded cystic disease as of low incidence; he had not intended to give that impression.

With regard to delayed ovulation, work done in this country showed that delayed ovulation would be a herd problem, but in view of the fact that he was treating the subject as a discussion of infertility in the individual cow, he had not thought it played a very important part in individual cases of infertility.

In reply to Mr. Brown, if one diagnosed a pregnancy at 5 weeks it at any rate gave some idea as to what was happening and whether in actual fact possibly early abortion or foetal resorption was taking place.

On the question of the use of iodine solution in the treatment of pyometra, he stressed that it was a procedure in which one must adopt a great deal of care to avoid any damage to the cervix or the uterus.

On cystic disease, Mr. Jennings had said that he found it difficult to say when cystic disease began. During the course of some investigations that he had himself done involving repeated examination of the ovaries of cattle within the first 2 months after parturition, he had observed that in very many cases during that period apparently cystic bodies developed and that these later disappeared. But on one examination one would have been inclined to say that the animal had an ovarian cyst. Most of these cases, however, cleared up spontaneously.

He thanked Mr. Pasfield for the very able way in which he had opened the discussion and had filled in many of the blanks in the paper. He certainly agreed with Mr. Pasfield about the need to preserve a due sense of humility in attempting to recognise and differentiate between structures in the ovary. In the paper he had included a small table which he regarded merely as a guide. He found it extremely difficult to decide on the nature of any cysts which might be present; one could form some opinion as to their probable nature but one could never be dogmatic.

He agreed entirely with Mr. Pasfield on the question of anoestrum in heifers. One found this much more frequently in animals which were poorly fed and managed, and in many cases it was largely a question of poor nutrition and bad management.

On the diagnosis of pregnancy, he felt that this was probably very much a personal matter, and what might be appreciable to one person was not necessarily appreciable to another person to the same degree.

He had been interested in the high degree of endometritis in Mr. Pasfield's practice, and he thought that this bore out what was said in the paper, namely, that one could not argue from one area to another. What appertained to one area was not necessarily typical of what appertained to another area; nor was it possible to go from one farm to another and expect the same conditions and the same results.

Mr. Pasfield had drawn attention to the enucleation of the corpus luteum, and said that the degree of risk was low. The risk was very low indeed, and he would not like anybody to think that it was of a high degree. Nevertheless there is a risk and it should always be borne in mind.

The Chairman proposed a vote of thanks to the author of the paper and the opener of the discussion.

I. The Diagnosis of Johne's Disease in Cattle and the Identification of *M. johnei* Infection

BY

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SUMMARY—1. The microscopical examination of faeces samples has a marked positive value in clinical disease, but a negative result has no significance, and further investigation is necessary before the presence of isolated organisms in the faeces of non-clinical animals can be accepted as evidence of actual infection.

2. Reactions to allergic tests are most marked in the initial stages of infection and become less regular as the disease becomes established. This type of test has a greater experimental than practical value, because the interpretation of a positive result in the field is complicated by the occurrence of cross-reaction in any animal skin sensitive to avian tuberculin.

3. The complement fixation test is the most generally useful of the recognised diagnostic methods, but this test also has its limitations. It is of no value in the very early stages of infection, and from experience in certain herds its use for the identification of infection in the absence of other evidence appears to be complicated by a degree of non-specificity.

4. The complement fixation test has a marked practical value for the confirmation of suspected disease. It also makes it possible to send for slaughter a large number of infected animals whilst they are in good bodily condition.

5. It has a limited value for sale or export certification, inasmuch as its use will prevent the exchange of most animals with unapparent but established disease; but it will not identify very early infection, and it appears that some non-infected animals may react. It is of no value if the animal is less than one year old, and any certificate should be for the absence of complement-fixing antibodies rather than a guarantee of freedom from infection.

6. There is insufficient evidence to justify the use of this test at present on a herd basis for eradication. The initial promise of the experimental work on this application of herd testing has not been maintained.

7. Herd testing has some possible application to control. Regular elimination of reactors does prevent clinical disease, and does therefore considerably reduce the dissemination of infection. The practical economic use of the test for this purpose is limited. It is most satisfactorily applied to dual-purpose or beef herds where the early disposal of reactors for meat represents little economic loss. Its use in this respect is more debatable in pedigree and dairy herds where every animal has an intrinsic value.

8. In view of the commonly-held opinion that the majority of infection takes place in calfhood, there is a special application of the test to any form of husbandry involving suckling or nurse-cows. A blood-negative status of the animals in direct contact with calves would reduce the chances of infection.

9. Attention is drawn to familial susceptibility to infection, and its importance to any diagnostic procedure aimed at eradication.

Introduction

CLINICAL Johne's disease is not an invariable sequel to infection with *Mycobacterium johnei*. There is no doubt that infection may be present in an animal or in a herd in the absence of past or impending clinical disease; many infected animals never become clinical cases. In considering diagnostic methods, it is therefore necessary to distinguish between infection and affection; tests of practical value for the one may be of little or no use when applied to the other. Diagnosis of infection is really the cardinal problem, because any infected animal is a possible future clinical case; but a reliable means of diagnosing early clinical disease also requires some consideration because it is a valuable aid to differential diagnosis which eliminates unavailing treatment, may make possible the slaughter of an animal before loss of condition, and reduces the opportunity for the dissemination of infection. The ideal of diagnosis before the animal is capable of transmitting infection may be more difficult to attain, and it is very possible that for this purpose a combination of diagnostic methods or some hitherto undiscovered test will be found necessary.

In Europe and Asia, and perhaps elsewhere, there appears to be a widespread infection of the cattle population with *M. johnei* but, for reasons unknown, clinical disease develops only in certain herds. In the absence of vaccination or any general policy of eradication, diagnosis is of major importance to herds with clinical problems, but it must not be overlooked that the transfer of an infected animal to a different herd is commonly followed by a rapid breakdown, independently of the existence of clinical disease in the herd of origin.

Diagnosis, therefore, occupies a dominant position in any approach to the Johne's disease problem.

Laboratory Diagnostic Methods

These have recently been reviewed by Hole (1958) and include three types of test: faeces examinations, allergic reactions, and serological tests. Reference in this paper will be made only to recent work.

Faeces examinations have until very recently been recommended solely in the cases of suspected clinical disease when the animal is scouring. Doyle (1956) considered that only 25 to 30 per cent. of cases were

identifiable by this means at the first examination, and emphasised the importance of the typical clump of the organisms and the need for care when considering the significance of scanty single acid-fast organisms. The chief advantage of this method is its positive accuracy; the presence of one typical clump is accepted as indisputable evidence of lesions in the bowel wall, fragments of which are passed in the faeces of a scouring animal. The test has no negative significance, and the presence of one or two single acid-fasts in the faeces could conceivably, in the absence of other evidence, be attributed to ingested organisms passing through the bowel, if the environment is heavily infected. Cultural examination of the faeces is not only complicated by contamination problems, but is of little practical use for diagnosis because of the slow growth of the organism, and this procedure is reserved almost solely for experimental work. If faeces examinations are practical only in the scouring animal, as has been suggested, this test has only a limited diagnostic value, and has no direct application to eradication or control measures. Cunningham and Gilmour (1959) have described a new method of preparing and examining faeces smears, and their results suggest that this modification may enable a wider practical application of the faeces test.

Allergic tests have received more attention than any other diagnostic method. Intradermal tests with johnin and avian tuberculin have been the main subjects of study. Although in the United States some workers consider johnin to have a marked diagnostic value, its use in this country has not been much favoured in recent years. Reactions obtained in the natural disease have been disappointing and irregular, and Doyle (1956) attributes this to a low state of allergy rather than to a low potency of johnin. Rankin (1957) has found that in experimental infection an early response to avian tuberculin is invariably obtained, and this is also true after vaccination. In both these instances the initial dose of organisms is large. Stuart (1959) has also observed allergic reactions in calves after exposure to an infectious environment. The evidence therefore suggests that this reaction may regularly be obtained in the very early stages even if less apparent later. The practical use of this test is complicated by reactions being given by animals specifically or non-specifically sensitised to avian tuberculin, something of sufficiently common occurrence to confuse the Johne's interpretation. It is possible that some of this avian tuberculin sensitivity is due to *M. johnei*, but there is no evidence to suggest that an avian reaction in a bovine animal is necessarily an indication of Johne's disease.

Serological tests have long been recognised as having a possible application to the diagnosis of Johne's disease, but until comparatively recently have been neglected in favour of the more popular allergic investigations.

Complement fixation is the only test of this nature to have received sufficient attention to warrant consideration in this paper. Reports from this country, Europe, Iceland and New Zealand all agree that, while of no value for very early infection, this is the most reliable laboratory test available for most stages of the disease. Although not an ideal or wholly satisfactory test, it has a definite value for practical application to some aspects of diagnosis until it can be replaced by a better method. It will identify the presence of unsuspected infection, it has a marked value for the confirmation of suspected disease, and initial trials (Hole, 1956) suggested it might be found to have some application to eradication.

The test is not capable, however, of identifying the early stages of infection. Rankin (1957) found that in experimental disease a reaction might be obtained as early as 3 months after infection, but Weybridge experience with a large number of tests on infected herds would indicate that in natural infection a true positive reaction in an animal of less than 9 months of age is exceptional, and the commonest time for the initial reaction is between 1 and 2 years of age. Reactions seen under natural conditions in very young animals are almost certain to be the result of a passive transference of antibody from an adult animal via the milk. Furthermore, the occurrence of temporary reactions in apparently normal animals has still to be satisfactorily explained and the practical specificity of the test conclusively demonstrated.

Specificity. Although a test with some degree of non-specificity may be of practical value applied on a herd basis, many dairy herds are not strictly herds but collections of individuals, and test results are assessed on an individual basis. Under such circumstances greater emphasis is placed on the importance of non-specificity, and this, therefore, requires careful consideration in relation to diagnostic value.

Some measure of non-specificity is to be expected in serological tests for Johne's disease antibodies, due to infections with closely related mycobacteria; but it is important to collect precise scientific evidence, and not to assume that the presence of another mycobacterium explains the reaction to the Johne's disease test. The possible existence of plurality of infection must not be overlooked, something clearly demonstrated by the Agricultural Research Council Committee Report (1941), where 11 out of 30 tuberculous animals were found also to have Johne's disease.

The only acceptable criterion of cross-reaction or non-specificity is to produce a reacting serum by administering the suspected antigen to a known non-reacting bovine animal; the reaction of a Johne's serum with a non-specific antigen might be due to the presence of multiple antibodies, a possibility which would have to be eliminated by complicated absorption tests. One of the saprophytic acid-fast bacilli (sometimes accidentally introduced into the bovine udder) has been shown to stimulate the production of a serum antibody which will react with the Johne's antigen to fix complement (Stuart, 1959); a parallel titration with the homologous antigen gives a higher (specific) titre. Infections of this kind in the field are relatively rare, however, and unlikely to account for a large measure of non-specific reactions. The same can be said of cross-reactions with bovine tuberculosis, as the test is being used on an attested cattle population in this country. The measure of possible interference by the avian tubercle bacillus is more difficult to assess, but it is by no means certain that more than a fraction of the avian tuberculin sensitivity is the result of avian infection, and experience does not

suggest any consistent correlation between avian tuberculin reaction and complement-fixation tests for Johne's disease.

Some animals with lesions of "skin tuberculosis" react; many do not, and it must not be assumed that, because some degree of skin sensitivity to tuberculin is attributed to such lesions, the same holds good for the Johne's complement-fixation test. There seems very litle doubt that the antibody concerned with skin sensitivity is not the same as that involved in complement fixation.

Apart from these recognised hazards, tests carried out on a herd basis in the absence of history of the disease have in a number of instances revealed an unexpectedly large number of reactors. It has been contended that the complement-fixation test is too non-specific to have a practical general herd application. Much of the evidence supporting this view is inconclusive, because the possible existence of occult infection has not been investigated, but an exception is the report by Rankin (1958). This covers the repeated use of the complement-fixation test on two groups of animals, one comprising a herd in which clinical disease is recognised, and the other three herds considered to be free from infection. The average incidence of reactions to a number of tests over 2 to 3 years on the animals in these two groups was 19 per cent. in the infected and 14 per cent. in the non-affected, a difference so small as to suggest a marked degree of non-specificity, especially when coupled with the incidence of temporary reactions. This conclusion is not wholly supported by the further evidence provided in this report. All animals removed from these herds have been regularly examined post-mortem, and of 94 such animals from the infected group 20 per cent. yielded a positive blood at slaughter, as compared to only 4 per cent. of the 351 animals from the non-infected group. This fivefold difference and the low figure for the non-infected group does not suggest marked non-specificity. The discrepancy between the percentage of positives found during life and at slaughter is explained by Rankin on the grounds that the slaughtered animals are old and not a random sample, the inference being, although data are not given, that non-specific reactions occur mainly in younger animals and are of a temporary nature. While this may be a correct explanation, it is difficult to see why it does not apply to both groups; the infected group showed 20 per cent. of reactions in both *ante-* and *post-mortem* tests, whereas in the non-infected group the reactions were reduced from 14 per cent to 4 per cent.

In the same report the low incidence of reactors obtained in tests on Irish cattle (4 per cent.) is attributed to the very low incidence of *M. johnei* infection in Eire. There seems to be no reason why non-specific reactions should occur only in cattle in contact with the Johne's organism.

The percentage figures reported by Rankin are exceptionally high. At Weybridge the testing of *known affected* herds over several years has given lower overall figures; in 1952 14 per cent. of reactions were obtained from 6,829 tests, in 1953 12 per cent. from 17,177, in 1957 and 1958 9·5 per cent. from 13,602 and 7,300 tests respectively, and overall only 11 per cent. of reactions were obtained at the first test of 28,540 animals from infected herds. Nevertheless, in the few herds with a reliable disease-free history that have been tested, a marked diversity in results has been recorded, from 0 per cent. to, in one isolated instance, 25 per cent. of reactors. It is noteworthy that up to the present, in every instance in which material has been made available, Weybridge has been able to prove infection in one or more animals from all herds in which complement-fixation reactions have been obtained, but the number of supposedly disease-free herds involved is very small.

The great obstacle to any assessment of non-specificity is the difficulty of proving the absence of infection. Johnson (1959) recently mentioned lesions and organisms confined to the upper end of the duodenum. Smith (1958) suggests the retropharyngeal lymph nodes as portals of entry. There is no guinea-pig to assist in detecting occult infection as in tuberculosis, and it would appear to be necessary, for a definite negative diagnosis, to examine by histological and cultural methods the whole alimentary tract and associated lymph nodes—an impractical procedure.

It must be concluded, therefore, that although circumstantial evidence points to the occurrence of a considerable percentage of non-specific reaction in herds with no clinical history of disease, there is insufficient evidence to prove beyond doubt that all such reactions are unconnected with *M. johnei* infection. It seems probable that this problem will not be satisfactorily elucidated until more light is thrown on the differentiation of *M. johnei* infection and Johne's disease, a distinction suggested by Taylor (1949, 1952), Rankin (1954) and Smith (1954).

Experimental Complement-fixation Tests and Related Investigations at Weybridge

Methods of diagnosis are applicable to individuals or to herds. In Johne's disease, tests on individuals have two main objectives: either the confirmation of suspected disease or the certification of freedom from disease for sale or export purposes. Tests on a herd basis have been made either to assess the extent of infection, or to attempt to eradicate or control the disease by the elimination of reactors. The complement-fixation test has been experimentally used for all these purposes for some years at Weybridge.

Tests on Individual Animals

Additional data collected have confirmed the original findings (Hole, 1952, 1953) on the value of this test as a means of confirming suspected disease. Specimens from 2,258 animals have now been examined and checked with the blood test findings; 85 per cent. of the 1,640 positive specimens were from blood-positive animals, and 9 per cent. from blood doubtfuls. Although 89 per cent. of blood-positive animals yielded positive specimens, this specificity figure must be treated with reserve, since the specimens were from clinically suspected animals, and five times as many infected as non-infected have been received.

Tests on a Herd Basis

The failure to identify very early infection makes a

single herd test no more than a very rough guide to the extent of infection, although experience does suggest it picks out the most likely immediate candidates for future clinical breakdown. Herd testing has been applied mainly as a repetitive procedure aimed at attempting to eliminate infection from herds with a Johne's disease problem. This experiment was originally planned on the hypothesis that the scouring animal was the prime disseminator of infection; if repeated testing and elimination of reactors prevented the occurrence of clinical disease the exposure of oncoming stock would be steadily reduced and infection might well ultimately be eliminated. The essential conditions have been regularity of testing and removal of reactors. This work has been dependent on the co-operation and goodwill of owners, and because of the constant conflict between experimental and practical requirements it has been necessary to test a large number of herds to obtain satisfactory data from a few in an experiment lasting five years or more. Although it does not at present appear to be succeeding in its primary object, experimental herd testing in general has provided considerable important secondary data that would not otherwise have become available.

The results of this experiment can be summarised as follows:

1. A policy of testing and elimination of reactors has, with very few exceptions, prevented the occurrence of clinical disease.

2. The early effect of repeated testing was a steady overall reduction in the number of positive blood reactors. This was not consistent in every herd; a few responded very well (Hole, 1956), a few very badly. Heath (1957) reported that the effect of five repeated tests on 54 herds was to reduce positives from 8 per cent. at the first test to 1 per cent. at the fifth test, and the Weybridge findings broadly agreed.

3. Continued testing has not, however, resulted in an increased number of these herds becoming reactor-free. There have been numerous instances of an inexplicable "resurgence" of reactors.

The apparent failure of persistent testing and elimination of positives permanently to clear herds of blood reactors infers either that the eradication of clinical disease does not materially lessen the chances of infection, or that many of the positive test results are non-specific reactions. This conclusion has led to subsidiary investigations, two of which are outlined below.

1. The Positive Value of the Test Applied to Preclinical Disease

Preliminary investigation has shown that, applied to herds with a clinical problem, the test had a marked positive value. Of the 29 blood reactors identified at the first test on 3 such herds, 27 became clinical within 4 years (Hole, 1955). Further evidence was obtained from specimens received from reputed non-clinical blood-positive animals, 42 of the first 50 received showing evidence of Johne's disease. Van der Schaaf and Zantinga (1955) reported 20 per cent. of blood-positive "normal" cattle as post-mortem negative. The occasional occurrence of numerous reactors in herds with no history of disease and the unsatisfactory herd testing results have led to a more detailed investigation being undertaken; clinically normal but blood-positive cattle are being examined for the presence of *M. johnei* by post-mortem examination at Weybridge.

The interim results of this investigation emphasise the need for examining an adequate number and representative sample. Of the first 25 animals examined, 21 showed evidence of Johne's disease and one of bovine tuberculosis; examinations of the next 25 animals have failed to reveal evidence of *M. johnei* or other acid-fast organism in 18 instances. A point of considerable interest which will require further investigation is that the first 25 animals were almost all obtained from herds with a current Johne's disease problem, whereas the second group of 25 were obtained largely from herds which have been testing and eliminating reactors for some years. This may be coincidence, and it must not be assumed that repeated testing and elimination will result in the disappearance of demonstrable infection even if reactors persist. For example, in one herd that has been testing for 5 years 42 animals have been slaughtered; lesions and organisms have been demonstrated in 36, lesions in 5, and no evidence of disease obtained in one instance only.

A larger number of animals must be submitted to post-mortem examination, and further investigation is necessary of the occurrence and significance of apparently non-infected blood reactors, including some animals taken from herds with a long history of testing and elimination.

2. Familial Susceptibility

Although not strictly a problem of diagnosis, reference to this aspect is made in relation to the apparent failure of herd testing to eliminate all infection despite its ability to prevent clinical disease.

Recent evidence obtained at Weybridge suggests that some cattle families are much more susceptible to infection than are other families of the same breed kept under identical conditions on the same farm. A similar difference has been noted in certain lines within families. Attention has so far been focused on the female lines, but it is possible the male may also be implicated.

One example will suffice to explain the position at the present stage. A large, long-established herd with a husbandry of suckling, nurse cows and group rearing of calves has been regularly blood tested for 6 years. This procedure stopped clinical disease, but has not permanently reduced the incidence of reactors. Recent investigation has shown that of the herd's 27 families 7 (including some of the larger and more successful) have had no clinical case or blood reaction; 7 other families have yielded not more than 2 isolated reactors, and 13 families have produced nearly 90 per cent. of the clinical cases and blood reactors. In most of these 13 bad families certain female lines have been exempt. The picture does not appear to be one of prenatal infection, as no clinical cases have been bred from for some years, and one or two generations have sometimes missed infection.

This requires further investigation. Experimental

evidence indicates that a considerable exposure at an early age is necessary for infection; circumstantial evidence in natural disease does not support such requirements, and the possibility of minimal exposure causing disease is corroborated by the frequency of the incidence on hygienic farms, the failure of calf segregation to eradicate, and the apparent spread of infection in the absence of clinical cases disseminating appreciable concentrations of organisms.

It is possible that eradication could be brought about by a combined policy of blood-testing and selective breeding. It is also possible that reconsideration will have to be given to the selection of animals for experimentation.

Future Herd Testing at Weybridge

The experimental investigation of the possibility of eradicating the disease by repeated testing is being wound up, except for a small nucleus of selected herds which are to be submitted to more frequent tests and more restrictive test conditions.

References

Agr. Research Council Comm. Rept. on Johne's Disease (1941). *J. Hyg.*, **41,** 297.

CUNNINGHAM, M. P., and GILMOUR, N. J. L. (1959). *Vet. Rec.*, **71,** 47.

DOYLE, T. M. (1956). *Vet. Rec.*, **68,** 869.

HEATH, G. B. S. (1957). *State Vet. J.*, **12** (36), 53.

HOLE, N. H. (1952). *Vet. Rec.*, **64,** 601.

——. (1953). *Proc. 15th Intern. Vet. Congr. Stockholm*, **1,** Pt. 1, 173.

——. (1955). European Product Agency, Paris. Project **207,** 91.

——. (1956). *Brit. Vet. J.* **112,** 273.

——. (1958). *Advances in Vet. Science.* Academic Press, New York, Vol. 4, 341.

JOHNSON, H. W. (1959). U.S. Dept. Agric., Beltsville. Personal communication.

RANKIN, J. D. (1954). *Vet. Rec.*, **66,** 550.

——. (1957). Thesis No. 625, Reading University.

——. (1958). *Vet. Rec.*, **70,** 383.

SMITH, H. W. (1954). *J. Path. Bact.*, **68,** 367.

——. (1958). Ibid., **76,** 201.

STUART, P. (1959). Central Veterinary Laboratory, Weybridge. Personal communication.

TAYLOR, A. W. (1949). *Vet. Rec.*, **61,** 539.

——. (1952). *Vet. Rec.*, **64,** 603.

VAN DER SCHAAF, A., and ZANTINGA, J. T. (1955). *Tijdschr. Diergeneesk.*, **80,** 1002.

II. Incidence of the Disease

BY

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SUMMARY—The term "incidence" as used in disease surveys is defined. Incidences of clinical Johne's disease in dairy cows recorded in surveys by the Veterinary Laboratory, Weybridge, and in investigation by the Milk Marketing Board in recent years are given.

Breed incidences based on the limited material available are recorded, and an estimate of approximately 0·6 per cent. as the probable level of clinical disease in England and Wales is made by correlation with breed distribution in the national herd. Some corroboratory data from knackery statistics are quoted.

Recent work on the isolation of M. johnei *from abattoir cattle, which suggests that about 11 per cent. of apparently normal animals may be carriers, is summarised.*

UNFORTUNATELY, the term "incidence" is often used loosely. If employed as a technical term, it should be clearly defined. A suitable definition would be: "the rate or extent of a disease or condition in a population, usually expressed as a percentage". The population also needs definition, whether the ordinary population of a given species or class of animal in a county, region or whole country, or a specialised section—e.g., animals disposed of, sent to abattoirs or knackeries, or to laboratories for examination.

In the case of Johne's disease, with a prolonged incubation period and a comparatively small proportion of animals harbouring the organism that become clinical cases, a careful distinction must be made between the incidence of clinical disease and of infection or latent disease. A proportion of infected animals undoubtedly never exhibit active symptoms.

Cattle: Incidence of Clinical Disease

Apart from the early age of probable initial infection, sometimes pre-natal, it is generally agreed that clinical disease is commonest in animals 2 to 5 years old (Doyle and Spears, 1951), and a large proportion of losses are among dairy cows. Recent surveys have shown the incidence in cows in the second and subsequent lactations to be nearly twice that in first calvers.

Two series of surveys of wastage and disease in dairy herds have been carried out by the Veterinary Laboratory in recent years and the results, so far as clinical Johne's disease is concerned, are shown in Table I. Farmers or herdsmen were asked to keep records of deaths, disposals and sickness, and periodical visits were made by veterinary personnel to check and record the information, which is therefore "second-hand". Some cases recorded as Johne's disease may not have been due to this cause; on the other hand, a number may have been missed by accident or concealment and recorded as disposed of for other reasons. All cases recorded by farmers, many of them confirmed clinically by veterinary surgeons, all those where laboratory tests were positive, and cases which appeared to be definite on questioning, were included.

In the Surrey, Berkshire and Wiltshire series of surveys, over 200 herds participated for varying periods, many of them continuing to keep records for 4, 5 or 6 years. Altogether 755 herd-years, covering nearly 26,000 cow-years were observed. There were 217 cases of Johne's disease, an overall incidence of 0·84 per cent. In these samples 17·6 per cent. of the herds were of Channel Islands breeds, reflecting the high proportion of these breeds in the cow populations (Surrey 37 per cent.; Berkshire 23 per cent.; Wiltshire 9 per cent.) compared with 7·9 per cent. in England and Wales as a whole. The recorded cases occurred in 19·5 per cent. of the herds, but 30 per cent. of the Guernsey herds and 25 per cent. of Jersey herds recorded cases compared with 17 per cent. of herds of other breeds. The overall incidence was undoubtedly influenced by the high proportion of

TABLE I

INCIDENCE OF CLINICAL JOHNE'S DISEASE IN TWO SERIES OF SURVEYS

	Surrey, Berks and Wilts. 1950/1–55/6	Eleven surveys England (9) Scotland (2) 1953/4–56/7
Total herds in samples	755	1,110
Total cows	25,975	29,844
Mean size (cows)	34·4	26·9
Number of affected herds	147 (19·5 per cent.)	87 (7·8 per cent.)
Cows in these	5,735 (22·1 per cent.)	2,808 (9·4 per cent.)
Mean sizes (cows)	39·0	32·3
Johne's disease (cases)	217	126
Overall incidence	0·84 per cent.	0·42 per cent.
Incidence in affected herds	3·77 per cent.	4·49 per cent.

Channel Islands cows. Breed incidence will be considered in a later section.

The second series of surveys was carried out in 4 years, 1953–54 to 1956–57, in a much wider field. Three consecutive samples were surveyed in Devonshire, 2 each in Wiltshire, Shropshire, Ayrshire and Lanarkshire and 1 in Yorkshire (West Riding). Finally, a composite sample in Berkshire, Buckinghamshire, Oxfordshire, Gloucestershire, Northamptonshire and Warwickshire was surveyed by the Animal Health Division. These 11 samples covered 1,110 herds with a population of approximately 30,000 cows. There were 126 cases of Johne's disease, an overall incidence of 0·42 per cent. Only 87 herds (7·8 per cent.) recorded cases. The proportion of Channel Islands cattle in this series was approximately 4 per cent., only about half the average for the whole country. The incidence in the 2 Scottish samples (0·14 per cent.) was markedly lower than in the 9 in England (0·52 per cent.) and only 5·3 per cent. of herds were affected in the former compared with 8·4 per cent. in the latter. It is not suggested that these differences necessarily indicate such disparity between the mean incidences of the disease in the two countries, as the surveys in Scotland were confined to Ayrshire and Lanarkshire. It is, however, almost certainly due to the overwhelming proportion of Ayrshire cattle and may well be characteristic of the dairying areas of south-west Scotland.

Two investigations by the Milk Marketing Board also provide data on the disease in dairy herds. A survey of wastage in Private Milk Records herds since 1955–56 covering approximately 1,000 herds with a cow population of about 20,000 cows per annum has recorded an incidence of 0·4 per cent. in the 3 successive years ending 1957–58 (Stewart and O'Connor, 1957, 1958; O'Connor, 1959). A more extensive inquiry in 1957 (Bownass and Smith, 1958) by means of a questionnaire obtained information from over 6,000 herds in 22 of the Board's centres widely distributed in England and Wales with an overall incidence of disposals on account of Johne's disease of 0·21 per cent.; but the range in particular areas varied from 0·03–0·52 per cent. It appears, however, that the population on which the incidences were calculated (totalling over 190,000) included in-calf and bulling heifers and the incidences in cows only would probably be higher than those recorded. This was a one-visit inquiry at which farmers were asked what stock they had lost during the preceding year, and a number of cases may well have been missed through faults of memory or by concealment. The affected herds were 321 or 5·1 per cent. of those visited.

Incidence in Affected Herds

As already mentioned, the proportions of affected herds in the various samples have varied from about 5 to 20 per cent. It may be that density of cattle population, locality factors and breed distribution are involved in the variable incidence of infection. It is of some interest that, irrespective of the percentage of herds affected, the *mean* incidence in such herds seems to be fairly constant, viz.: from 3 to 5 per cent. Thus, in the Surrey, Berkshire and Wiltshire samples it was 3·8; in the 11 samples 4·5 (England, 4·75; Scotland, 2·83) and in the M.M.B. investigation 4·1 per cent. On the other hand, individual herds have shown wide variation. Data from the Weybridge series of 11 surveys which have been analysed in some detail show

TABLE II

BREED INCIDENCES OF CLINICAL JOHNE'S DISEASE IN ELEVEN REGIONAL SURVEYS

Breed	English samples (9)			Scottish samples (2)			All samples (11)		
	Cows	Cases	Per cent.	Cows	Cases	Per cent.	Cows	Cases	Per cent.
Friesian	8,546	23	0·27	175	0	—	8,721	23	0·26
Shorthorn	4,249	34	0·80	0	0	—	4,249	34	0·80
Ayrshire	3,322	9	0·27	7,492	11	0·15	10,814	20	0·18
Guernsey	803	8	1·00	2	0	—	805	8	0·99
Jersey	314	15	4·78	5	0	—	319	15	4·70
Others	4,858	26	0·54	78	0	—	4,936	26	0·53
All breeds	22,092	115	0·52	7,752	11	0·14	29,844	126	0·42

TABLE III

ESTIMATED INCIDENCE OF CLINICAL JOHNE'S DISEASE FOR ENGLAND AND WALES BASED ON NINE ENGLISH SAMPLES AND BREED COMPOSITION OF NATIONAL HERD

Breed	Proportion of cows per cent. national herd	Breed incidence per cent.	Per cent. of national herd affected
Friesian	40·6	0·27	0·110
Shorthorn	25·3	0·80	0·202
Ayrshire	18·3	0·27	0·049
Guernsey	5·3	1·00	0·053
Jersey	2·6	4·78	0·124
Others	7·9	0·54	0·043
Estimated incidence per cent. in national herd			0·581

that, of 87 herds which recorded cases, there were 68 with one case, 12 with 2 each and only 7 having 3 or more cases up to a maximum of 8 in one herd. Mean herd incidence in those with one case was 3·2 per cent., in herds with 2 cases 6·0 per cent., and in herds with 3 or more cases incidences varied from 9·5 to 13·3 per cent.

Here it may be mentioned that results of the Ministry's first field trial for 11 years (1946 to 1956 inclusive) show the incidences of diagnosed and suspected cases in vaccinated and unvaccinated stock to be 0·43 and 4·02 per cent. respectively (Report on Animal Health Services in Great Britain, 1957). These figures are very close to the incidences found in surveys for whole populations and in affected herds only.

Breed Incidences

In the Surrey, Berkshire and Wiltshire series of surveys, the incidence of the disease in Channel Islands cows was 1·95 per cent. (Jersey, 2·11 per cent.; Guernsey 1·88 per cent.), compared with an average of 0·60 per cent. for all other breeds.

The series of 11 surveys have been analysed for breed incidence in detail and the results are given in Table II. The incidence was much higher in Jerseys than in all other breeds. The combined figure for the two Channel Islands breeds (23 cases, 1,124 cows; 2·05 per cent.), is closely similar to that in the Surrey, Berkshire and Wiltshire surveys. There was some difference between the incidence in Ayrshires in the samples in England (0·27 per cent.) and those in Ayrshire and Lanarkshire (0·15 per cent.), but no significance can be attached to this.

These figures are presented objectively as the findings and it is appreciated that they are based on small numbers of clinical cases and, in the case of the Channel Islands breeds, the total number of cows under observation was not large. There were no significant differences between the incidences in the various areas in England, but there was a highly significant difference between the incidence in Jerseys and that in other breeds.

In Table III an assessment has been made of the probable mean incidence for England and Wales by correlating breed incidences in the 9 English samples with the distribution of breeds in the national dairy herd (M.M.B. 1955 census).

Knackery Data

In the English samples in the second series of surveys, 21 per cent. of the cows disposed of to knackeries were cases of Johne's disease. Based on a return of one quarter's receipts at knackeries in England and Wales and the relative proportions of animals disposed of owing to disease at different seasons, the total knackery intake of cows is estimated at approximately 80,000 to 90,000 per annum. This would suggest that about 18,000 cows (0·7 per cent. of the national dairy herd of 2½ million) are disposed of annually on account of this disease.

Surveys by Phillips and Hughes (1952), Norval (1954) and Peck (1957) have also indicated that between 20 and 30 per cent. of knackery cattle may be affected with Johne's disease. It is estimated that about 150,000 cattle of all ages may be disposed of annually to knackeries in England and Wales and 35,000 to 40,000 are therefore probably cases of Johne's disease. Relating this estimate to total cattle of all ages (8,190,000), or on the assumption that the clinical disease is confined to animals over 2 years old (4,600,000), the incidence would be 0.5 or 0.8 per cent. respectively.

Incidence of Latent Infection

Adult cattle. During the last 10 years several workers (Taylor, 1949, 1952; Rankin, 1954; Smith, 1954) have cultured the ileo-caecal lymph nodes from samples of apparently normal cattle slaughtered at abattoirs, and *Mycobacterium johnei* has been isolated from variable numbers ranging from 6 to 17 per cent. More recently, Smith (1958) has reported the examination of 100 adult abattoir cattle from which the organism was obtained in 7 cases, but there was a higher incidence in the retro-pharyngeal than in the ileo-caecal nodes. He concluded that the pharyngeal area may be an important portal of infection. The results of these assessments of latent infection are summarised in Table IV, which shows that in the aggregate 11·2 per cent. were positive, and suggests that the "carrier" animal may be approximately 15 to 20 times as common as the clinical case.

TABLE IV

CULTURAL EXAMINATION OF ABATTOIR CATTLE FOR THE PRESENCE OF *Mycobacterium johnei*

Worker	Number of animals (cattle)		Per cent positive
	Examined	Positive	
Taylor (1949)	154	22	14·3
„	89	15	16·9
„ (1952)	665	101	15·2
„	136	8	5·9
Rankin (1954)	552	32	5·8
„	358	27	7·5
Smith (1954)	300	51	17·0
„ (1958)	100	7	7·0
Total	2,354	263	11·2

Calves. Chandler (1959) examined the ileo-caecal lymph nodes of 447 calves up to 2 months old from Birmingham abattoir and isolated *M. johnei* in only 2 cases (0.45 per cent.). Cunningham and Gilmour (1959) have carried out a similar investigation on 158 calves under one week old in the Glasgow area and only one was positive (0·63 per cent.). In neither case was the pharyngeal region examined and if this had been done it might have increased the number of positives. Nevertheless, an incidence of infection in calves of approximately 0·5 per cent. is indicated and this *may* represent the incidence of congenital infection as suggested by Rankin (1959).

Incidence in Sheep

Information available on Johne's disease in sheep is meagre, and until surveys are carried out the incidence remains a matter for conjecture. Annual loss in affected flocks has been reported at up to 10 per cent. (McEwen, 1939; Doyle, 1956). Smith (1954) cultured the organism from only one of 200 abattoir

sheep in an area in which the disease was uncommon. On the other hand, Heath (1955) demonstrated acid-fast organisms indistinguishable from *M. johnei* in intestinal smears of 46 of 214 abattoir sheep (21·5 per cent.) in an area where the disease is common. Loss of condition in sheep is almost invariably attributed to parasitic infestation, and when Johne's disease occurs it is usually overlooked and may become established before it is recognised (Doyle, 1956).

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References

BOWNASS, R. C., and SMITH, G. F. (1958). M.M.B. Prod: Div. Rep. No. 8., 1957-58, p. 129.
CHANDLER, R. L. (1959). *Vet. Rec.*, **71,** 147.
CUNNINGHAM, M. P., and GILMOUR, N. J. L. (1959). Ibid., **71,** 295.
DOYLE, T. M. (1956). 74th. Brit. Vet. Assoc. Congress, Leamington.
DOYLE, T. M., and SPEARS, H. N. (1951). *Vet. Rec.*, **63,** 355.
HEATH, G. B. S. (1955). 73rd Brit. Vet. Assoc. Congress, Belfast.
MCEWEN, A. D. (1939). *J. Comp. Path.*, **52,** 69.
MINISTRY OF AGRICULTURE, FISHERIES AND FOOD (1959). *Rep. Animal Health Services in G.B., 1957,* p. 42.
NORVAL, J. (1954). *Vet. Rec.*, **66,** 508.
O'CONNOR, L. K. (1959). Private Communication.
PECK, E. F. (1957). *Vet. Rec.*, **69,** 939.
PHILLIPS, R., and HUGHES, L. E. (1952). *Brit. Vet. J.*, **108,** 458.
RANKIN, J. D. (1954). *Vet. Rec.*, **66,** 550.
——. 1959. Ibid., **71,** 332 (letter).
SMITH, H. W. (1954). *J. Path. and Bact.*, **68,** 367.
——. (1958). Ibid., **76,** 201.
STEWART, A., and O'CONNOR, L. K. (1957). *Vet. Rec.*, **69,** 1021.
——. (1958). M.M.B. Prod. Div. Rep. No. 8, 1957-58, p. 76.
TAYLOR, A. W. (1949). *Vet. Rec.*, **61,** 539.
——. (1952). Ibid., **64,** 603.

III. Vaccination against Johne's Disease: The Results of a Field Trial Experiment

BY

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SUMMARY—Vaccination of cattle against Johne's disease has been widely practised in France for more than thirty years and on a limited field trial scale in Great Britain since 1946. The type of vaccine used was originally developed by Vallée and Rinjard in France and consists of living M. johnei *suspended in oils. When inoculated subcutaneously, it causes a fibrous nodule to form at the site of inoculation which may persist for years. It is difficult to assess its value under laboratory conditions, but experience in the field strongly suggests that it confers a useful level of protection in the majority of herds. It may on occasions produce excessively large nodules, especially after revaccination, but its most serious disadvantage is that it sensitises animals strongly and persistently to both avian and mammalian tuberculins.*

WHEN cultures of living *Mycobacterium johnie* are inoculated into susceptible animals by the subcutaneous route, they do not appear to cause a progressive infection and set up the typical clinical disease. Two French scientists, Vallée and Rinjard (1926), were the first to take advantage of this to develop a vaccine which has become widely used in France to control the disease in cattle. The vaccine consists of a suspension of living *M. johnei* in a mixture of olive oil and liquid paraffin to which traces of fine pumice powder are added. After subcutaneous inoculation into animals, it sets up at the site a hard, fibrocaseous nodule which may remain palpable for years; it also sensitises them strongly and persistently to both avian and mammalian tuberculins. Experience in France and Great Britain has shown that the vaccine is safe, but positive evidence to confirm its protective power has been lacking. The chronic nature of the disease and the absence of any small susceptible animal which could be used in large numbers are two factors involved which have discouraged full-scale laboratory tests; the use of bovine animals or goats in sufficient numbers makes such tests both prolonged and expensive. Furthermore, another problem is to challenge experimental animals in the laboratory in circumstances which closely simulate those occurring under natural conditions; too severe a challenge may break down a level of protection which, though perhaps poor by other standards, might be adequate under field conditions. On the other hand, a challenge which is too weak may not produce enough clinical disease among the controls.

Doyle (1945) was the first to use the French type of vaccine in Great Britain and, after its safety was established, the A.R.C. started a field trial in 1946 in which 121 infected herds eventually took part. All of these herds were selected on a history of regular losses from the disease over a number of years, and the results were to be judged solely by the incidence of clinical disease in them over a long period after vaccination commenced. From the time of entry into the trial, all calves born into these herds were vaccinated before they were one month old, this age limit being fixed in order to minimise the risk of vaccinating already infected animals. No control animals were retained and animals older than one month at the time of entry were left unvaccinated to act as normal reservoirs of infection. Losses among unvaccinated animals were recorded but they cannot be regarded as controls, and as the trial progressed, their numbers, of course, gradually diminished as vaccinated animals matured and replaced them. The site of vaccination chosen was the side of the brisket where it was considered that the vaccinal nodule would be least obtrusive. Revaccination at intervals of 18–24 months was recommended, but this recommendation was in many instances overlooked and a considerable proportion of the experimental animals were vaccinated only once. The Ministry's divisional veterinary officers were responsible for supervising the herds in their respective counties, and at the end of every year submitted a return showing the numbers of vaccinated and unvaccinated animals present in the herds and the number of clinical cases of Johne's disease which occurred among them during the year. Animals which became clinically suspect were checked, where possible, by the laboratory examination either of faeces samples or post-mortem material; but owing to the failure by some owners to notify suspect cases, laboratory confirmation is lacking in a considerable proportion. These were listed as suspected cases in the annual returns; but to avoid complicating the tables presented here, confirmed and suspected cases have been added together.

The field trial has now been in progress for thirteen years and of the original 121 herds which entered it, 47 remained at the end of 1957, the last year for which complete figures are available. The absence of control animals confuses the exact interpretation of results, but the general opinion among both owners and their veterinary surgeons is that the vaccine was of considerable value in reducing losses. Overall losses were certainly reduced, but, as opinions may vary as to the exact role the vaccine played in this outcome, the results are presented in some detail in Tables I and II.

In Table I are set out the annual losses from Johne's disease among both vaccinated and unvaccinated animals and also, where known, the losses which occurred prior to entry to the trial. In the last column of this table, the overall losses are expressed in terms

TABLE I

Year	Number of field trial herds in which annual losses from Johne's disease were recorded	Losses among vaccinated stock	Losses among unvaccinated stock	Total losses	Average annual loss per herd
1941	67	—	150	150	2·2
1942	75	—	205	205	2·7
1943	83	—	272	272	3·3
1944	91	—	302	302	3·3
1945	102	—	349	349	3·4
1946	107	3	271	274	2·6
1947	106	1	281	282	2·7
1948	102	12	283	295	2·9
1949	92	3	208	211	2·3
1950	84	8	164	172	2·0
1951	80	19	114	133	1·7
1952	74	36	74	110	1·5
1953	64	27	40	67	1·0
1954	56	23	21	44	0·8
1955	54	17	18	35	0·6
1956	52	30	12	42	0·8
1957	47	11	4	15	0·3
		190	2,768	2,958	

of the average loss per herd per year, no differentiation being made between losses among vaccinated and those among unvaccinated animals. It will be seen that, over a period of 18 years, the average loss per herd gradually diminished from a peak of 3·4 animals per herd in 1945 to 0·3 animals per herd in 1957 and in the terms in which the object of the field trial was defined at its commencement, the vaccine would appear to have been remarkably successful. In Table II, the information is given in more detail and covers the years in which the trial was in progress; the reduction in overall losses is again reflected in figures expressed as percentages.

Miss C. N. Hebert, of the Central Veterinary Laboratory, has kindly analysed the results obtained over an eight-year period 1950–57 in the 47 herds which remained in the trial at the end of 1957. She has provided a short statistical analysis and comment which are reproduced in Table III. As will be seen, the analysis confirms the trends shown in Tables I and II.

TABLE III

Percentage loss among total stock (means of 47 herds)

1950	1951	1952	1953	1954	1955	1956	1957
2·228	1·606	1·591	0·909	0·796	0·743	0·502	0·304

Variation:

	d/f	*Mean Square*	*F*	$P_{0·01}$
Between herds	46	14·244	6·6	1·0
,, years	7	20·247	9·4	2·3
Error	322	2·159		
Total	375			

i.e., differences between herds and different years are highly significant.

In general, comparisons of mean values for different years show that the percentage losses show a significant drop over periods of 3–4 years.

Merely as a matter of interest, the 190 breakdowns are shown in Table IV, indicating, where known, the ages at which primary vaccination was done, the age

TABLE II

Year	Number of herds	Total vaccinated stock	Total unvaccinated stock	Total stock	Cases of Johne's disease among vaccinated stock	%	unvaccinated stock	%	total stock	%
1946	66	558	3,523	4,081	3	0·54	123	3·49	126	3·09
1947	82	1,504	4,637	6,141	1	0·07	167	3·60	168	2·74
1948	85	2,527	3,915	6,442	12	0·47	193	4·93	205	3·18
1949	90	3,527	4,274	7,801	3	0·09	195	4·56	198	2·54
1950	84	4,276	2,883	7,159	8	0·19	164	5·69	172	2·40
1951	80	4,821	2,163	6,984	19	0·39	114	5·27	133	1·90
1952	74	4,824	1,843	6,667	36	0·75	74	4·02	110	1·65
1953	64	5,320	1,277	6,599	27	0·51	40	3·13	67	1·02
1954	56	4,664	1,040	5,704	23	0·49	21	2·02	44	0·77
1955	54	4,790	1,053	5,825	17	0·35	18	1·71	35	0·60
1956	52	4,699	1,297	5,996	30	0·64	12	0·93	42	0·70
1957	47	4,381	909	5,290	11	0·25	4	0·44	15	0·28
					190		1,125		1,315	

TABLE IV

Analysis of 190 breakdowns

Age when first vaccinated		Age at breakdown		Number of times vaccinated	
At 1–7 days old	38	At less than 1 year old	3	Vaccinated once only	65
„ 8–14 „ „	27	„ 1 year old or over	16	„ twice	73
„ 15–21 „ „	33	„ 2 „ „ „ „	20	„ three times	11
„ 22–28 „ „	20	„ 3 „ „ „ „	40	„ four „	4
„ 29–35 „ „	14	„ 4 „ „ „ „	26	„ five „	1
At over 35 days old	14	„ 5 „ „ „ „	32	Number of times not known	36
Age not known	44	„ 6 „ „ „ „	8		
		„ 7 „ „ „ „	8		
		„ 8 „ „ „ „	4		
		Age not known	33		
	190		190		190

at time of breakdown and the number of times these animals were revaccinated.

In a few herds, the results were somewhat disappointing and losses among vaccinated stock still continue. Such herds were exceptional and the reasons for these failures are not clear, but it is possible that a particularly heavy ambient infection, low standards of husbandry, hygiene or nutrition, and perhaps misuse of the vaccine, may all have played a part. Some herds, but not many, experienced trouble with the formation of excessively large nodules, especially after revaccination. This appeared to be confined to certain herds, and in some instances the owners withdrew their herds from the trial, as they felt these large nodules seriously blemished their animals.

However, the most serious drawback to the vaccine is undoubtedly its sensitising effect to tuberculins. As already stated, it induces a strong and persistent sensitivity to both avian and mammalian tuberculins. Hebert, Doyle and Paterson (1959) selected 18 attested herds from those which took part in this trial and analysed their tuberculin test histories. They found that vaccination raised the reactivity to both tuberculins to about eight times that for the normal attested cattle population. In about 80 per cent. of tests, the reaction to avian tuberculin was 1 mm. or more than that to mammalian tuberculin, and in these instances the test interpretation was relatively straightforward; but in 15 per cent. of tests, the level of reactivity to mammalian tuberculin was equal to, or greater than, that to avian tuberculin. In these cases, interpretation is rendered much more difficult. Tuberculous infection could be masked by this non-specific sensitisation and the authors concluded that the use of vaccine is clearly contra-indicated in attested herds. This view is supported by the experimental evidence presented by Ritchie and Muir (1952) and by Doyle (1953).

Vaccination also influences the interpretation of the complement fixation test for Johne's disease diagnosis. Hole (1959) states that, in theory, successful vaccination is bound to stimulate the production of some sort of antibody and that in practice the amount or type of antibody produced seems to vary because some vaccinated animals develop a reaction to Weybridge complement fixation test and some do not. This inconsistency apparently makes it impossible to assess the significance of a positive complement fixation reaction in vaccinated animals.

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References

DOYLE, T. M. (1945). *Vet. Rec.*, **57**, 385.
——. (1953). *Brit. Vet. J.*, **109**, 275.
HEBERT, C. N., DOYLE, T. M., and PATERSON, A. B. (1959). *Vet. Rec.*, **71**, 108.
HOLE, N. (1959). Personal communication.
RITCHIE, J. N., ROBERTSON, A., and MUIR, R. O. (1952). Personal communication.
VALLÉE H., and RINJARD, P. (1926). *Rev. gen. Med. Vet.*, **35**, 1.

IV. Experimental Infection

BY

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SUMMARY—Experimental Johne's disease is discussed against the background of past and present experience. The paper describes some recent published and unpublished work on the subject. The estimation of an L.D.$_{50}$ dose of Mycobacterium johnei *for calves is described and the possible role of heredity in determining the relative susceptibilities of individuals within the same species is discussed.*

Mycobacterium johnei as an Infectious Agent

MYCOBACTERIUM johnei was seen by Johne and Frothingham in association with an enteritic disease of cattle in 1895 only a few years after Koch had discovered the tubercle bacillus, yet, when compared with tuberculosis, progress in the understanding and control of Johne's disease has been very slow. Among the reasons for this delay have been the lack of a small laboratory animal suitable for use in its study and the uncertainty of setting up the disease even in the natural hosts. This paper discusses the subject of experimental Johne's disease against the background of past and present experience.

Today it is probably still true to say that no useful technique has been evolved for the study of Johne's disease in laboratory animals. No condition even remotely resembling Johne's disease has so far been produced experimentally in the guinea-pig, rat, cat, Syrian hamster or the domestic fowl. Better results have been obtained using the rabbit, the mouse and the field-vole, although none of these animals can yet be regarded as completely suitable for Johne's disease investigation.

The first successful infection was reported by Bang (1914), who inoculated a rabbit *subcutaneously* with material from a para-tuberculous goat. He killed the rabbit 1¼ years later and found the small intestine to be thickened and packed with acid-fast bacilli. No evidence of tuberculosis was found and the rabbit was reported to be "in excellent condition". A remarkable feature of this report is that it is the only record of Johne's disease having resulted from a subcutaneous inoculation *in any species.*

It was Alikaeva (1940) who really set the pattern for the infection of laboratory animals with Johne's disease. He administered cultures of *M. johnei* to rabbits intravenously or by mouth, at the same time fasting them to lower their resistance. It is stated in the translation of his paper that intestinal lesions were "rare", from which it is fair to assume that they did occur. Lesions, however, were not seen until 12 months after inoculation, and Alikaeva states that the probable reason for the failure of earlier attempts to affect small animals was the premature termination of the experiments.

Rankin (1958) inoculated 8 two-week-old sucking rabbits with 2·5 mg. wet/wt. *M. johnei* by the intravenous route. Five of them developed intestinal lesions of Johne's disease and 3 of these were showing clinical evidence of the disease at the time of death. The incubation period was protracted, being not less than 7 months and usually about 1 year.

Francis (1943) inoculated mice of 14 days of age intraperitoneally with 2·5 mg. *M. johnei* and killed them from 9 to 34 weeks later. He found a progressive infection commencing in the liver and spleen and eventually involving the whole intestine. Lominski, Cameron and Roberts (1956) studied the problem more fully and successfully established infection in more than 100 mice. Thirty-eight were subjected to detailed histological examination, and intestinal lesions of the type seen in cattle were found in all of them. Mice of 2–3 weeks of age were used and they were kept for at least 1 year after infection.

The field-vole (*Microtus agrestis*) appears to have been used by only one worker; Levi (1950) produced intestinal lesions in 17 of 21 voles, inoculated intraperitoneally when young. with as little as 0·001 mg. wet/wt. of *M. johnei*. He also fed 8 voles with 10 mg. wet/wt. of bacilli and 5 of them became infected. The voles were kept for at least 1 year after infection. Because of the small dose of organisms required, the fact that it could with success be given orally, and the high proportion of "takes", Levi suggests that the vole is the animal of choice, and this may well be so, but no one has attempted to repeat his work.

The very early attempts to infect cattle experimentally met with a large measure of success. Bang (1906), Meissener and Trapp (1910) and Meyer (1913) succeeded in causing Johne's disease by administering infected bowel mucosa and lymph node suspension to calves. Meyer, however, noted that 2 of the older animals (one 6 months and the other 12 months) remained free from the disease. Some of the later attempts, however, were not so successful; Twort and Ingram (1913) were able to infect less than half of their experimental cattle and M'Fadyean and Sheather (1916) after 13 failures out of 22 attempts concluded that . . . *"it has to be noted as a remarkable fact that in the majority of cases the attempt to infect with Johne's disease failed"* . . . M'Fadyean and Sheather do not mention the age of their experimental cattle.

McEwen (1939) fed 6 heifer calves with infected material from sheep. The first dose was given when the calves were a few days old and 7 subsequent doses were given during the next ten months. All 6 calves developed the disease. Hagan (1938), also using the oral route, dosed cattle with 20 g. of infected intestinal mucous membrane. Of 9 calves under one

year old 7 became affected, 3 out of 3 dosed when between 1 and 2 years old became affected but none of 3 cattle dosed when over 2 years old became affected.

Both Taylor (1953) and Doyle (1953) have investigated this question of the relationship between age and susceptibility. Taylor failed to produce Johne's disease in any of the 4 calves infected when 6 months old, while at the same time producing the disease in calves infected at birth and at 3 months of age. Doyle dosed 12 cattle, aged 17 months to 6 years, with 20 g. infected intestinal mucous membrane on 3 successive days. Four of the animals showed varying signs of infection, but only 2 of the youngest developed the classical disease. The remaining 8 remained healthy.

Sheep and goats have also been successfully infected and in both these species a similar hardening of resistance with increasing age has been noted. McEwen (1939) caused Johne's disease in 8 of 9 sheep fed as lambs with *M. johnei* and stated that ... *"The disease is produced with considerable regularity in animals fed as sucking lambs with large quantities of the bacteria in the material from cases of Johne's disease. No adult sheep fed with similar material ... has become infected."* Two facts worthy of special attention appear in this short statement: (1) the disease was produced with regularity when sucking lambs were used; (2) no adult sheep became infected.

Levi (1948) succeeded in setting up Johne's disease in 18 of 25 goats fed as kids with infected intestine or with culture. He failed to cause the disease in 2 adults and concluded that adults were resistant.

It now seemed clear that the disease was being produced most frequently when the experimental animals were infected at an early age. Bearing this in mind, a series of infection experiments on cattle was started at Compton in 1952. Of 6 one-month-old calves inoculated with 100 mg. wet/wt. *M. johnei*, 4 developed the disease 11 to 39 months later (Rankin 1958a). One of the two calves which remained healthy was shown to have considerable complement-fixing antibody to *M. johnei* in its serum at the time of infection and for some months later. This antibody had probably been received passively from the mother's colostrum.

For various reasons, other one-month-old calves have from time to time been inoculated intravenously with 100 mg. wet/wt. of *M. johnei*. On analysis I find that of 39 calves so treated roughly one-half developed Johne's disease in clinical form and 29 (75 per cent.) showed lesions of the disease at post-mortem examination. In every case, whether lesions were found or not, *M. johnei* was cultivated from the lymph nodes.

This apparently heavy dose of 100 mg. *M. johnei* has been diluted in tenfold steps and each dilution inoculated intravenously into one-month-old calves (Rankin, 1959). Cases of Johne's disease occurred in animals receiving 100 mg., 10 mg. and 1 mg. of the bacilli, but no calf receiving 0·1 mg. or less developed the disease during the period of the experiment. From the results of this experiment, it has been possible to calculate the $L.D._{50}$ of the strain of *M. johnei* used to be 5 mg. of the bacilli weighed wet.

Experiments using mature cattle instead of calves have given very different results. Six three-year-old cows were inoculated intravenously with 100 mg. wet/wt. of *M. johnei*. When they were killed four years later none showed macroscopic evidence of Johne's disease. All, however, were still harbouring the bacillus which was recovered in culture from the lymph nodes.

Two and a half years ago a group of 12 cows, all over 4 years old, were given a course of intravenous inoculations of *M. johnei* during studies of antibody production. A dose of 5 mg. was given and some of these cows have now had no less than 37 separate doses. All the cows have calved once, and some twice since the inoculations began, and so far none has shown any evidence of Johne's disease. All have given positive avian tuberculin tests and complement-fixation tests.

Some work has also been done on the fate of the Johne's bacillus when inoculated intravenously into cattle (Rankin, 1957). Calves, inoculated at one month old with 100 mg. wet/wt. of *M. johnei*, were killed after 1 week, 1 month, 3 months, 7 months, 10 months and 18 months. Cultural and histological examinations of an extensive selection of the organs and lymph nodes were made. As one would expect, the lungs acted as a filter and a large number of the inoculated bacilli, particularly those in clumps, were held back in the capillaries within the alveolar walls. The lungs of calves killed one week after inoculation contained many such trapped bacilli but the mildness of the cellular reaction was noteworthy. One month after inoculation the bacilli could no longer be seen in the lung microscopically, but they were recovered in culture. Damage to the lung tissue was minimal and consisted of thickening of some alveolar walls and increase in the number of macrophages which could well have resulted from other causes. The organism could still be cultivated from the lung three months after inoculation, although histologically the lung was normal except for a few small foci consisting of macrophages.

Throughout the period 1 week to 3 months after inoculation *M. johnei* was cultivated from a large selection of tissues including lung, liver, spleen, kidney, intestine, uterus, bladder and many lymph nodes. No histological evidence of change was found in these organs until the 3-month period when small groups of endothelioid cells were seen in the hepatic lymph node. It is perhaps worth noting that the first true lesions seen were in lymphoid tissue and not in the intestinal mucous membrane.

It was 7 months after inoculation before the first intestinal lesions were found. These consisted of distension and distortion of the villi in the ileum as a result of endothelioid cell infiltration and proliferation. A few giant cells were also now in evidence.

From 7 months onwards variation was only one of degree and recovery of the organism was possible from a wide selection of lymph nodes and organs with the exception of the heart muscle, which was consistently negative. Many of the lymph nodes showed lesions of the endothelioid cell/giant cell type and the intestinal lesions sometimes progressed until almost all matrix cells had been replaced by endothelioid cells.

In none of the cases here referred to, nor in any

of my other cattle experimentally infected with *M. johnei*, did the disease process advance beyond the stage of proliferation and infiltration. Necrosis, caseation or calcification were never seen.

One unexpected result of the method of infection by culture intravenously was that some calves did not grow well. There was no obvious sign of ill health, but the end result was a heifer, or cow, of small proportions. This may have been the result of the initial invasion of the lungs but the small degree of histological change found would suggest some other cause. Clinical disease is often produced earlier by this method than would be expected in the field and it may be that extensive intestinal involvement with consequent diminution in functional ability occurs before the animal has grown to its full size.

Infection by contact has also been tried with some success at Compton. Cattle, known to be excreting *M. johnei* in their faeces, were yarded on straw in order to create an infectious environment. Nine calves, varying in age from 1 week old to 6 months old, were kept in this environment either until they became clinical cases of Johne's disease or were killed 4½ years later. When the experiment was ended 6 of the 9 calves had developed intestinal lesions of Johne's disease. Two of these affected animals were 6 months of age when introduced to infection for the first time. At post-mortem *M. johnei* was cultivated from the lymph nodes of all 9 animals.

A similar experiment involving adult cows is in progress at the present time. Seven 4-year-old cows were introduced to an infectious environment in 1956 and have been kept there to date. At the same time 7 control calves were put in the yard in order to establish that infection was indeed present. Faeces samples from the cattle in both groups have been cultured for *M. johnei* at monthly intervals. For the first 14 months all samples were consistently negative; then positive cultures began to appear in the control group of animals. At the time of writing (27 months after first exposure) all control animals have been excreting *M. johnei* in their faeces and one has died of clinical Johne's disease. Three others are in subnormal bodily condition and look as if they may develop the disease in clinical form. Throughout the same period no sample of faeces from any of the adult cattle has yielded a growth of *M. johnei*. One cow, however, became thin and began to scour about 20 months after exposure. Numerous faeces samples were examined microscopically for *M. johnei* with negative results and complement-fixation tests were also negative. As the diarrhoea did not abate the cow was slaughtered. Post-mortem examination showed the intestine to be free from obvious lesions such as corrugations, but the jejunum might have been slightly thickened. The ileum was paper-thin throughout its length and the caecum and rectum also appeared to be normal. Histological examination of the intestine revealed numerous acid-fast bacilli in the jejunum and ileum and an increase in the number of lymphocytes, polymorphs and eosinophiles. Macrophages were present in considerable numbers, but did not occur in groups or as a symplasma. A few giant cells were seen. The caecum and rectum were normal and no acid-fast bacilli were seen in these sites. The other 6 cows in this group remain in good condition and it is intended to keep them in this environment for a further 2 years.

A number of other infection experiments involving mice, rabbits and cattle are in progress at Compton, but it is too early to say very much about them. Dr. Chandler and I are trying to work out suitable doses of *M. johnei* for the experimental infection of calves by the *oral* route and Dr. Payne is studying the pathogenesis of the disease caused by bacilli administered by this route. This work is by no means complete, but it is interesting to note now that, immediately after dosing, the tonsilar tissues were found to be heavily invaded by the bacilli. This would appear to be another piece of evidence in favour of the portal of entry of the bacillus being lymphatic rather than by way of the intestinal lumen.

Comment

The early experiments using *M. johnei* as an infectious agent were designed simply to demonstrate that disease could be produced in a certain species or type of animal and the appearance of the disease was regarded as the highly successful end-point. As a result of these early experiments, it became clear that, by using young cattle and keeping them for a long time, Johne's disease could be expected in a high proportion of cases. The obvious next step was the establishment of something in the nature of a Minimal Infective Dose and/or an $L.D._{50}$. This, however, was not possible either physically or economically at that time and has had to await the present more liberal attitude to veterinary research and the creation of an institute, such as Compton, where large animals can be properly maintained in numbers suitable for the determination of such basic facts. This, in turn, has brought its own problems, the greatest of which is that of the supply of animals of the right type. Now you will quite rightly ask "What is the right type?" and I shall have to admit that I do not know. At present we have been feeling our way in this and have settled for the best that is available to us at the moment.

So far in all my experiments I have used cattle of the Ayrshire or Ayrshire/Red Poll cross breed (one or two Friesians have sometimes had to be included to make up numbers) and my results therefore apply to such cattle, but not necessarily to, say, the Shorthorn or Channel Island breeds. Only animals showing no reaction to either avian or mammalian tuberculin are used and their sera must have no complement-fixing antibodies for *M. johnei*. We know that calves can be born already infected with the Johne's bacillus (Lawrence, 1956), and that a high proportion of apparently normal adult cattle harbour the organism (Taylor, 1949). We also know that the majority of such animals may pass the above tests. How, then, can they be excluded from the experiments? I believe that at present the only way to do this is to draw the experimental sample from a closed population in which no such infected animal exists. To this end I have, during the past 6 years, examined culturally samples of the mesenteric lymph nodes of the majority of adult cattle disposed of for any reason from 2 dairy herds where clinical Johne's disease is not known to

exist, and in no case has *M. johnei* been cultivated. Over 200 cattle have been dealt with in this way and it is estimated that had the organism been present in these herds, as it is in the general cattle population of Britain, it would have been recovered on 12 to 34 occasions. Where possible, therefore, experimental animals are drawn from these 2 herds.

Recently it has been shown that the recovery rate of *M. johnei* from the lymph nodes of young calves is very small indeed, being less than 1 per cent. (Chandler, 1959; Cunningham and Gilmour, 1959), and Cunningham and Gilmour (loc. cit.) suggest that such calves would form a useful source of experimental animals with a low incidence of infection. With this I cannot, at present, agree for reasons already stated (Rankin, 1959a).

In spite of this care in selecting experimental cattle free from all previous contact with *M. johnei* and other pathogenic mycobacteria, a considerable proportion of the animals in some experiments have failed to succumb to the infection. This raises the question of individual variations in susceptibility and the possibility that these may be transmitted genetically. Mr. Hole has told us in his contribution to this symposium almost all that is known about this aspect of Johne's disease. By analogy with tuberculosis, however, we can consider this possibility further.

Lurie (1952) has demonstrated a remarkable difference in the susceptibility to human-type tubercle bacilli between 2 strains of rabbit. This difference is passed on constantly from generation to generation and can be defined anatomically as the possession by the susceptible strain of an enlarged adrenal cortex, and physiologically as the possession of an increased amount of circulating cortisone. There is no reason why similar anatomical and physiological variations are not inherent in certain breeds and strains of cattle, but the testing of such a hypothesis would be a formidable undertaking.

References

ALIKAEVA, A. P. (1940). *Veterinaryia, Moscow*, **2**, 26.
BANG, B. (1906). *Proc. Nat. Vet. Assoc.*, p. 125.
BANG, O. (1914). *Proc. Xth Int. Vet. Congr., London*, **3**, 157.
CHANDLER, R. L. (1959). *Vet. Rec.*, **71**, 147.
CUNNINGHAM, M. P., and GILMOUR, N. J. L. (1959). *Vet. Rec.*, **71**, 295.
DOYLE, T. M. (1953). *Vet. Rec.*, **65**, 363.
FRANCIS, J. (1943), *J. comp. Path.*, **53**, **140.**
HAGAN, W. A. (1938). *Cornell Vet.*, **28**, 34.
JOHNE and FROTHINGHAM (1895). *Dtsch. Z. Tiermed. Path.*, **21**, 438.
LAWRENCE, W. E. (1956). *Vet. Rec.*, **68**, 312.
LEVI, M. L. (1948). *J. comp. Path.*, **58**, 38.
——. (1950). *J. comp. Path.*, **60**, 10.
LOMINSKI, I., CAMERON, J., and ROBERTS, G. (1956). *J. Path. Bact.*, **71**, 211.
LURIE, M. B., ABRAMSON, S., and HEPPLESTON, A. G. (1952). *J. Exp. Med.*, **95**, 119.
M'FADYEAN, SIR J., and SHEATHER, A. L. (1916). *J. comp. Path.*, **29**, 62.
MCEWEN, A. D. (1939). *J. comp. Path.*, **52**, 69.
MEISSENER, H., and TRAPP (1910). Quoted by Twort and Ingram, 1913.
MEYER, K. F. (1913). *J. Med. Res.*, **29**, 147.
RANKIN, J. D. (1957). Thesis, Univ. Reading.
——. (1958). *J. Path. Bact.*, **75**, 363.
——. (1958a). *J. comp. Path.*, **68**, 331.
——. (1959). *J. Path. Bact.*, **77**, 638.
——. (1959a). *Letter. Vet. Rec.*, **71**, 332.
TAYLOR, A. W. (1949). *Vet. Rec.*, **61**, 539.
TAYLOR, A. W. (1953). *J. comp. path.*, **63**, 355.
TWORT, F. W., and INGRAM, G. L. Y. (1913). *A Monograph on Johne's Disease*, Bailliere, Tindall & Cox. London.

THE SPEAKERS' INTRODUCTION

The Chairman, Mr. S. L. Hignett, in introducing the authors, said that, owing to the importance of Johne's disease in the country at the present time, this was one of the outstanding scientific sessions of the Congress, certainly for those who were interested in the health of the farm livestock concerned.

The Chairman then introduced the authors and called upon them successively to present their papers.

Mrs. Mary H. Maclay, in presenting the paper by Mr. N. H. Hole and herself on "The Diagnosis of Johne's Disease in Cattle and the Identification of *M. johnei* Infection", said that the paper covered not only Weybridge experience with complement fixation tests, but also gave the authors' opinion of the practical value of other recognised diagnostic methods.

Mrs. Maclay then summarised the paper, and showed the following as lantern slides:

(1) A comparison of blood test results with microscopic examination of *post-mortem* specimens from cases of suspected disease.

Positive P.M. specimens			Negative P.M. specimens		
P.	D.	N.	P.	D.	N.
1,399	137	103	179	160	280
Total 1,639			Total 619		

SLIDE 1.—Comparison of blood test results with microscopical examination of *post-mortem* specimens from cases of suspected disease.

(2) Herd blood test results.

Year	Total	Positive	Percentage
1952	6,829	950	14
1953	17,177	2,134	12
1957	13,602	1,293	9·5
1958	7,300	696	9·5

SLIDE 2.—Herd blood test results.

(3) Five years' testing of an infected herd divided into its component families.

(4) The distribution of blood reactors in this herd.

(5) The results of *post-mortem* examinations done on some cows which were giving positive blood reactions but showed no clinical signs when purchased after reacting.

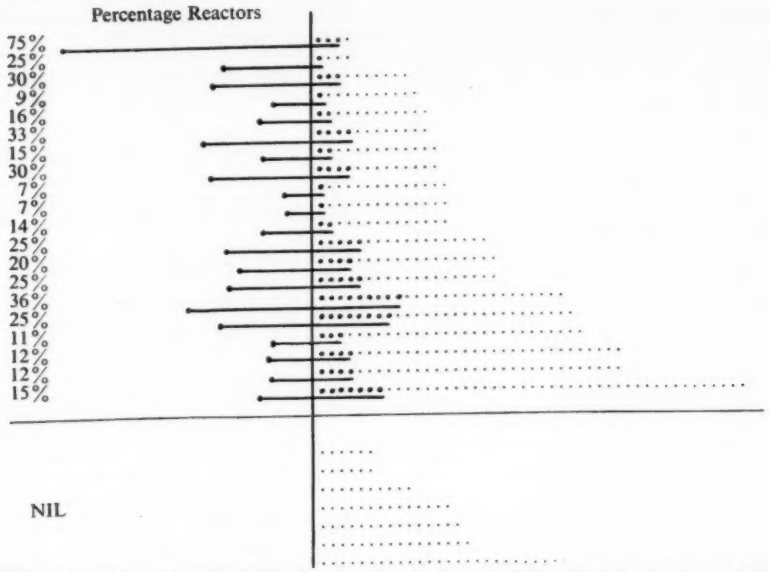
(6) Photographs (not available) of some of the animals covered in the table on page 1162.

Dr. F. W. Withers, in presenting his paper on "Incidence of Johne's Disease", said that this question was an important one, because until they had some idea of the general level of infection throughout the country they were really working in the dark, as with any disease in which the extent of the problem was unknown.

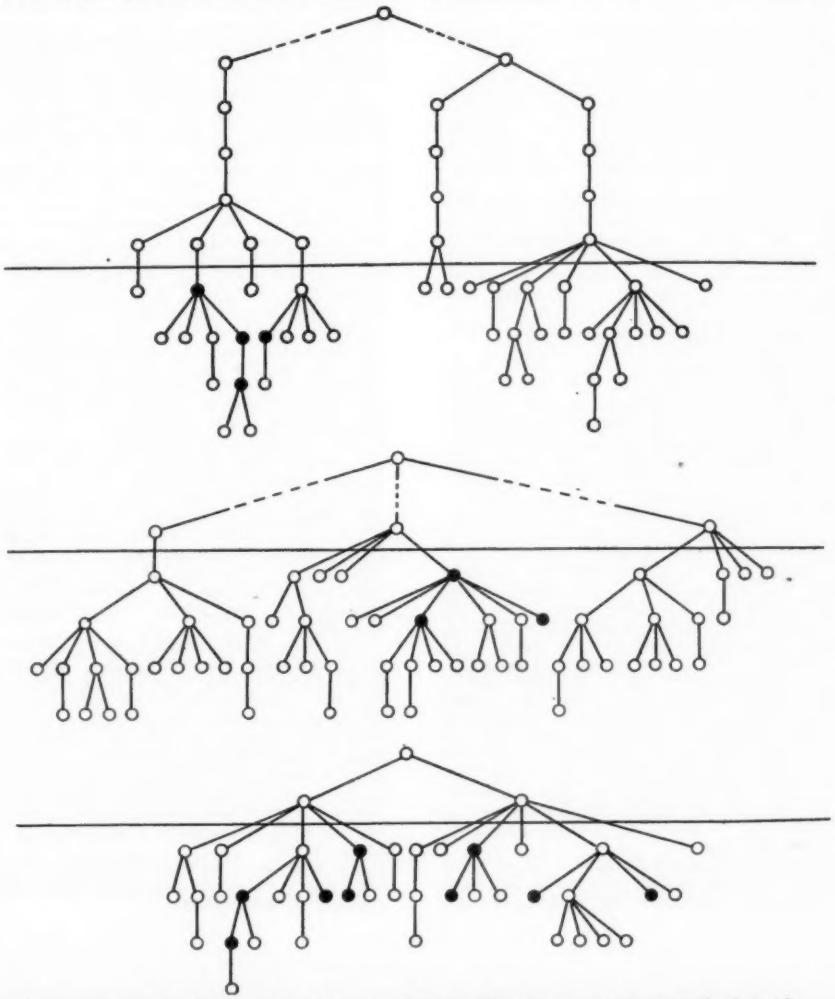
He did not regard himself as in any way an expert on Johne's disease. He came into the picture only incidentally, having for the past few years had something to do with the surveys in which information had been collected on quite a number of diseases, of which Johne's disease was one.

Following the preliminary surveys on which the paper was based, the Ministry's Animal Health Division had started in 1957 a national survey of dairy herds covering every county in the country, and some results for the first year, 1957/58, were now becoming available. Mr. Leech had let him have some information relating to Johne's disease. These figures were preliminary and might subsequently be subject to modification or revision, because they had been got out only recently.

It appeared that the size of the first year's national sample, which averaged roughly 1 per cent. of the dairy herd population of this country, contained 1,158 herds and some 29,000-



SLIDE 3.—Five years testing of an infected herd. Each horizontal is one family and shows relative size and percentage of blood reactors



SLIDE 4.—Three family trees (female lines). Reactors ● No test records above horizontals.

TISSUE	ANIMAL NUMBER																		
	802	825	990	822	924	**820**	733	736	**800**	767	**824**	1,055	**1,058**	769	3,173	823	**768**	948	821	925
Jejunum 1	+	+	+	+	+	+	+	+	+	+	−	−	+	−	−	+	−	−	−	−
Lymph node	+	+	+	−	−	+	+	+	−	+	−	+	−	−	−	−	−	−	−	−
Jejunum 2	+	+	+	+	+	+	+	+	−	+	−	−	−	+	+	+	−	−	−	−
Lymph node	+	+	+	+	+	+	+	+	−	+	+	−	−	−	−	−	−	−	★	−
Ileum 1	+	+	+	+	+	+	+	+	+	+	+	−	+	+	−	−	−	−	−	−
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−
Ileum 2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	★	−
I/C/Valve	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−	−	−
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	★
Caecum	+	+	+	+	+	+			+	−	+	+	+	+	+	+	−	−	−	−
Colon	+	+	+	+	+	−			+	−	+	+	−	−	+	−	−	−	−	−
Retropharyn Lymph node		−	+	−	−	−					−	−	−		−	−	−	−	−	−

★ = Histological lesions confirmed by culture.

SLIDE 5.—Microscopical examination of tissues from 20 non-clinical but infected blood reactors.

odd cows, almost exactly the same number as in the 11 preliminary surveys dealt with in the paper.

The total estimated incidence of diagnosed and suspected cases of clinical Johne's disease in cows for the whole country was 0.45 per cent., which was very close to the figure that had been obtained in the preliminary work, viz. 0.42 per cent. The proportion of affected herds was 7 per cent. in the national sample compared with 7.8 per cent. for the aggregated earlier figures.

The incidence in Jersey cows was much lower than had been found in the earlier surveys—only 0·83 per cent. compared with 4 7 per cent. Incidentally, in the national survey the figure in Guernseys was higher than that in Jerseys, whereas it had been the other way round in the earlier figures. The important point was that the incidence was significantly higher in the Channel Island breeds, and significantly lower in the Ayrshires, than in other breeds. This was in accordance with the previous findings.

With regard to regional incidence, it was lowest in the north of Scotland, and then followed the south of Scotland, Wales, north and south-east England, south-west England eastern England, and finally the west Midlands, which had the highest figure. The incidence in Scotland, especially in the north, was again significantly lower, and in the West Midlands significantly higher, than the average for Great Britain as a whole. He thought that he was justified in saying that the low incidence in Scotland was fairly obviously due to the large number of Ayrshires in their dairy herds in which the incidence was lowest amongst the breeds.

It therefore seemed that in a representative national sample one would find clinical cases of the disease in less than 10 per cent. of the herds, and yet from information that was obtained from the examination of abattoir material and other tests it seemed that *infection*, as opposed to *clinical cases*, was very widely disseminated. Taking the average, as given in the last paragraph of the paper, about 11 per cent. of over 2,000 abattoir examinations had yielded the organism. If they could accept that less than 10 per cent. of herds at any time had clinical cases, he thought that the important thing to find out was to what extent *infection* was disseminated. There was one method by which that could possibly be done. It was for those who examined material from the abbatoirs to get information as to the origin of the cattle and to ascertain whether the herds had a history of the disease.

It was suggested that there might be quite a lot of herds in the country with infected animals which did not have clinical cases. It seemed to him that sooner or later, if there was infection in a herd, one would get clinical cases. He thought it would be a valuable contribution to the problem if some information on this could be obtained.

Mr. H. N. Spears, in presenting his paper "Vaccination against Johne's Disease: The Results of a Field Trial Experiment", said that when one looked through the literature it was surprising how little experimental work had been done on the development of vaccines against such an important disease as Johne's. No doubt the nature of the disease itself explained this. Laboratory experiments must be carried out on large and expensive animals, and they had to be kept under observation for a long time.

Vallee and Rinjard's vaccine had been produced in France for over 30 years, and he believed that some 75,000 to 80,000 doses were used there annually. Unfortunately, they had very little positive field evidence as to its precise value in controlling the disease. Doyle at Weybridge had been the first to use the French vaccine experimentally in this country, and after he had shown it to be at least safe, the Agricultural Research Council Johne's Disease Technical Committee had initiated a field trial in 1946 and the results of that trial were set out in the paper.

The information forthcoming was meagre but there was no disputing the fact that there had been a gradual and steady decline in the overall incidence of clinical Johne's disease in the herds taking part. But, unfortunately, because there had been no control animals included, these results, when viewed by the cold eye of the statistician, might not be convincing. He himself, with some others who had worked with the trials for over 13 years, were convinced that the vaccine had played an important part in this reduced incidence, but their approach might be more emotional than scientific.

The critic of the results could point out many flaws. He could say that the incidence of clinical Johne's disease might fluctuate widely in a herd over a long period and that the reduced losses merely reflected a natural regression of the disease. Other factors, such as changes in husbandry, improved hygiene, or possibly change-overs to different breeds, might influence the results. It was difficult to ignore those criticisms but if any of these factors, or combination of them, had accounted for the reduced losses in these herds and the vaccine had played no part, then it was comforting to think that such simple measures could bring the disease under control.

The French vaccine which had been used had many disadvantages, the main one being that it sensitised animals strongly to tuberculins.

When the field trial had been planned, it had been designed to run parallel with and be complementary to a second experiment. That second experiment envisaged the purchase of a number of heavily infected herds and the vaccination of the calves reared, leaving a suitable number unvaccinated as controls. Unfortunately, the second experiment had never come to pass.

A second field trial had been started about 2 years ago, and they now had over 300 herds taking part. A proportion of the calves were being retained as controls, so that a proper statistical interpretation could be made in the end, but it would be a long time before the results could be analysed.

Stuart, at Weybridge, was carrying out a controlled vaccination test in cows, but again it would be some time before results would be available.

Finally, he wished to take the opportunity of paying a tribute to Mr. Doyle of Weybridge. Mr. Doyle had been his chief for many years and had pioneered much of the work on Johne's disease in this country. Mr. Doyle had been largely responsible for the initiation of the field trial and for much of the experimental work in connection with it.

Dr. J. Deans Rankin, in presenting his paper on "Experimental Infection", said that every time he rose to address an audience of veterinary surgeons on Johne's disease, he offered a little prayer for forgiveness. He always thought, "Here we go again, with the same old mixture as before, and no remedy at the end of it". In mitigation, he would point out that they were dealing with a very slowly progressive disease, and with a bacillus which required perhaps 3 months or 6 months to cultivate. This bacillus might also be on the borderline of being a pathogen.

The Chairman had intimated that it could be assumed that all those present had read the papers. He was going to assume, on the contrary, that they had not done so—for, after all, why should they have done so? They had come to the meeting to listen and to inquire; if they had only wanted to read the paper they could have stayed at home—and what with wine-tasting ceremonies and alumnus dinners, they would probably have had a better chance of reading it at home!

Dr. Rankin then read the section "Comments" from his paper.

The Opener

Dr. A. Marr, who opened the discussion, said that when he accepted the Provisional Committee's invitation to open the discussion on this symposium he did so with considerable trepidation as he realised that Johne's disease was not the easiest subject on which to talk. Had he realised just how difficult that task was going to prove he thought his trepidation might have reached the point of absolute refusal.

They had before them 4 very interesting and informative papers given by workers who had devoted many years, and it was obvious, considerable thought and talent, to the subject of Johne's disease. His experience of the disease was confined to its manifestations in the field. He would not attempt therefore to follow the authors into the deeper waters into which they had ventured but confined himself to an attempt to discuss the papers from the point of view of the light they threw on the disease as seen by the practising veterinary surgeon.

Dr. Withers had essayed the extremely difficult and perhaps somewhat thankless task of assessing the incidence of Johne's disease. The difficulties of assessing accurately the clinical incidence of a disease such as this were enormous and would, in some respects, seems to be insurmountable. But as Dr. Withers had pointed out, the results of different surveys were remarkably similar and it would be seem that his paper demarcated the extent of the problem as accurately as was possible.

Dr. Withers has produced some interesting figures on the breed incidence of Johne's disease. Dr. Marr realised how diffident collectors of data were about drawing conclusions from the material they had collected, but he would like Dr. Withers to hazard an opinion on the reasons for the apparent differences in incidence. He noted that he was careful to avoid at all times using the term "breed susceptibility" and spoke always of "breed incidence". However, in discussing the markedly lower incidence in the 2 Scottish as compared with the 9 English surveys he had stated that the disparity "is almost certainly due to the overwhelming proportion of Ayrshire cattle" in the Scottish sample. Did he then regard the Ayrshire as less susceptible than, say, the Channel Island breeds, or did he think, as seemed to Dr. Marr more likely, that the explanation was to be found in husbandry methods? In Scotland, and even among breeders of Ayrshires in England, it was common practice to remove calves from their dams at birth whereas, in the case of Channel Island breeds, it was the almost invariable practice to allow the calves to suckle their dams for at least 3 to 4 days, and even subsequently the young stock remained in much more intimate contact with the adults than was usual in Scottish herds. However, as Mr. Hole and Mrs. Maclay had pointed out there was good evidence of familial susceptibility and it was perhaps likely therefore that certain breeds as a whole were more susceptible. This was just one example of the equivocal nature of much of the evidence that they had before us.

In his analysis of the available data on the incidence of latent infection Dr. Withers had brought out clearly the very important fact that this might be 15 to 20 times as high as the incidence of clinical disease. This point was well brought out by Mr. Hole and Mrs. Maclay in the first 2 sentences of their paper when they said "Clinical Johne's disease is not an invariable sequel to infection with *Mycobacterium johnei*. There is not doubt that infection may be present in an animal or in a herd in the absence of past or impending clinical disease; many infected animals never become clinical cases". This was one of the most intriguing aspects of the whole disease and had led various workers to suggest that a sharp distinction should be drawn between Johne's disease and infection with *Mycobacterium johnei*. Many aspects of the disease, particularly perhaps the observation that movement from one farm to another might precipitate the clinical picture in the absence of clinical Johne's disease in the farm of origin, suggested that such a distinction might be a valid one. If they accepted such a concept it implied that some quite specific factors were involved in the transformation of infection with *Mycobacterium johnei* into affection with Johne's disease. They all recognised that the stress of calving and early lactation were important precipitating factors but apart from this they had little real knowledge. The elucidation of the factors involved in clinical breakdown would be a valuable step forward but their experimental investigation would present such formidable difficulties that is was unlikely to be rewarding.

Mr. Hole and Mrs. Maclay had given an exhaustive account of the present position regarding the diagnosis of Johne's disease and infection with *Mycobacterium johnei*. It would seem that the diagnosis of clinical Johne's disease by faeces' examination and the application of the complement fixation test was on a fairly satisfactory basis. He had found in a small number of cases that the improved technique for making faecal smears recently described by Cunningham and Gilmour (1959) was a distinct improvement on older methods but they did not yet know whether the method has any wider application than to the diagnosis of clinical disease.

The diagnosis of latent infection was much more complicated and here he must confess that he found it difficult to decide from the paper just how they should assess the value of the complement-fixation test. It seemed to him, however, that the weight of the evidence produced by Mr. Hole and Mrs. Maclay suggested that the test was fairly specific for *Mycobacterium johnei* infection and that such apparent anomalies as the occurrence of positive reactors in herds free from clinical Johne's disease did not necessarily indicate non-specificity.

Hole (1956) had suggested that recovery from clinical Johne's disease with regression of a positive complement fixation test, might occur. This would suggest that transient infection was quite likely and might account for many of the apparent anomalies which result when the test was applied on a herd basis. It would be interesting to hear from Mr. Hole what proportion of positive blood reactors in herds free from clinical Johne's disease were of a temporary nature.

They must all share Mr. Hole's and Mrs. Maclay's disappointment that the application of the complement-fixation test on a herd basis did not at present appear to be succeed-

ing in its object of eliminating infection from herds with a Johne's disease problem. They could not therefore at present be too sanguine about the possibility of the test being used as the basis of an eradication programme. Mr. Hole and Mrs. Maclay had however suggested that the test might have a practical application in herds with a severe Johne's disease problem in detecting the most likely candidates for future breakdown. It would seem to him that many owners of beef and dual purpose herds with such a problem, and their veterinary surgeons, would welcome the chance of having such information at their disposal. He would like to ask whether it was thought that the time had come for facilities for herd blood testing, on this limited basis, to be made available. If such facilities were made available by the Ministry, at the public expense, provision would have to be made for the marking of reactors and their exposure for sale, except for slaughter, forbidden.

Dr. Rankin had given them a very full and valuable account of the present knowledge on experimental infection with *Mycobacterium johnei*. His account of his attempts to establish precise information on a Minimal infective Dose and an L.D.50 would be very useful indeed to those engaged in Johne's disease research. From the point of view of the clinician, the part of his paper which was of the most immediate interest was that dealing with age susceptibility to infection. The great bulk of experimental evidence pointed to the difficulty of infecting older animals. He would like to hear Dr. Rankin's views on whether he thought that the same held good under field conditions. Hole (1955) had suggested that field evidence indicated that adult infection could not be ruled out. If this was so it had, of course, a great bearing on the possibility of control by segregation of young stock. His own view, so far as it was possible to interpret field evidence, was that adult infection was probably not an important factor. Perhaps Dr. Rankin and Mr. Hole could each give them their views on this question as it was one of great practical importance.

It would also be of great interest if Dr. Rankin could give them an account of his views on the reasons for the difficulty in infecting older cattle, under experimental conditions at least.

Although he realised that the question was of only academic interest at the moment, it would be very interesting if Dr. Rankin could give them some idea of the work which had been done on the sensitivity of *Mycobacterium johnei* to chemo-therapeutic agents, and whether he saw any likelihood of practical advances in the field in the foreseeable future.

Finally, Mr. Spears had given them an account of the Field Trial Experiment on vaccination against Johne's disease. As he had said, the absence of controls confused the exact interpretation of results. While it would appear that the overall incidence of clinical disease decreased with the use of the vaccine, there were so many other factors which might have played a part that it would be rather dangerous to draw any firm conclusions. The final assessment of the vaccine would have to await the result of the controlled trial at present in progress.

There were, however, one or two points in Mr. Spears' account on which it may be useful to comment. In his analysis of the 190 breakdowns in Table IV of his paper one found that a smaller proportion of breakdowns occurred in aimals vaccinated once only than in animals subjected to re-vaccination, and yet in the text of his paper Mr. Spears had told them that a considerable proportion of animals were vaccinated once only. It would appear therefore that re-vaccination did not have any beneficial effect and indeed it might be thought that re-vaccination increased the chances of breakdown. It would help them in trying to assess the situation if Mr. Spears could give them more precise information on the proportion of animals vaccinated only once.

It would also be of great interest if Mr. Spears could tell them whether breakdown to vaccination occurred sporadically throughout the vaccinated population or whether breakdown was high in particular herds and, if so, whether it could be correlated with any particular type of husbandry? He asked this because in their Sevenoaks practice they had had a high incidence of clinical Johne's disease in one particular dairy herd in which all calves had been vaccinated for the last 11 years and in which it had been the practice to rear the calves by suckling on nurse cows. These nurse cows had in the main been old cows discarded from the milking herd because of age or, more ominously, because of poor production. Some of these nurse cows had developed into clinical cases of Johne's disease and had been confirmed as such, so that they would understand that the vaccine had, to say the least, been subjected to a very severe test.

Despite the somewhat unusual circumstances something could be learned from the use of the vaccine in this herd. Firstly, single vaccination (not surprisingly) had failed to exercise any apparent control and losses from Johne's disease had continued. To take the worst example—out of one year's crop of 12 heifer calves, 7 developed Johne's disease with either their first or second calf and another of this group had recently shown loss of conditions and slight intermittent scouring which past experience told him would eventually result in frank Johne's disease, although faecal smears were as yet negative. Several of these animals had still had palpable vaccination nodules at the time of clinical breakdown.

Re-vaccination at 18-24 months of age was done last year on one batch of 8 heifers and so far none of these had broken down. Re-vaccination had however to be stopped as every animal developed a very large swelling at the site, which in the majority of cases went on to abscess formation, and caused a serious blemish apart from causing some generalised detrimental effect on health at the time the abscess was at its height. Incidentally, he had had experience of re-vaccination in only 2 herds, but in both abscess formation at the site of the second vaccination had been a problem. He felt that Mr. Spears passed somewhat lightly over this difficulty but he may have been unlucky!

Another point which he wished to make was that the positive diagnosis of Johne's disease in vaccinated animals could be a problem as in his experience it seemed to be difficult in most cases to demonstrate *Mycobacterium johnei* in faecal smears even in the terminal stages and even when the condition had been quite readily confirmed on *post-mortem* examination. The complement-fixation test could not, of course, be applied as it was frequently positive in vaccinated animals. It would appear that although the vaccine had not protected against clinical disease it had resulted in a suppression of the numbers of Johne bacilli being passed in the faeces. He would be very interested to hear whether any of the authors had noticed a similar difficulty.

Although the conditions in this herd might be somewhat grotesque the fact remained that single vaccination did not appear to have exercised any significant control. He understood from Mr. Bruford that similar trouble, though on a much smaller scale, had occurred in two other herds in the Sevenoaks practice in the years before he joined it. It was only when steps were taken to segregate the calves at birth that the problem disappeared. One was perhaps entitled to wonder just how much husbandry factors might have influenced the results of the Johne Vaccine Field Trial and, as he had said, it would be very helpful if Mr. Spears could give them some information on whether breakdown was associated with any particular type of husbandry.

In conclusion, he thought he could say without fear of contradiction that there was as yet no easy and complete answer to the problem of Johne's disease. It would be wrong, however, to take too pessimistic a view as much could be done to mitigate its effects. He personally had little doubt that in dairy herds losses from the disease could be kept to a reasonable level by segregation of calves at birth and by attention to sensible methods of hygiene designed to limit the spread of infection. In beef herds the problem was undoubtedly more difficult but here he thought that the application of the complement-fixation test might play a valuable part, even though complete eradication could not be achieved. The role of the vaccine was as yet impossible to assess; they must hope that the controlled trial at present in progress would show that it too had its part to play.

References

CUNNINGHAM, M. P., and GILMOUR, N. J. L. (1959). *Vet. Rec.*, **71**, 47.

HOLE, N. H. (1955). *State Vet. J.*, **10**, 2.

——. (1956). *Brit. Vet. J.*, **112**, 272.

The General Discussion

Dr. R. L. Chandler (A.R.C., Compton): "I should like to ask 3 questions of Dr. Withers. Firstly, what method of selection was used to choose the herds studied for their incidence of Johne's disease? I have myself made studies on the incidence of Johne's disease and know what a difficult subject it is. Very often the owners who are willing to co-operate in such surveys are those who are, by their good husbandry, most likely to keep the incidence of the disease as low as possible; conversely, the unco-operative farmer is often the one who tends to have Johne's disease on his property and to conceal the fact.

"Secondly, has Dr. Withers noted any correlation between the type of climate or the type of soil on the incidence of Johne's disease? I have noted areas overseas where there is at any rate an *apparent* relationship between climate—an area having fairly high rainfall and no dry summers—and the incidence of Johne's disease. Furthermore, workers overseas have in one case correlated incidence with one predominant soil type.

"Thirdly, I would like to ask Dr. Withers if he has any information regarding incidence in beef herds. The disease *can* occur in beef cattle but there is little information on its extent and importance.

"I have also some questions and comments to put to Mr. Spears about vaccination. Are the strains of *Mycobacterium johnei*, used in the standard vaccine, infective when inoculated intravenously or given orally to cattle—or have they become attenuated and avirulent with prolonged culture?

"Secondly, Mr. Spears mentioned that some critics of the field vaccine trial have postulated some "fluctuation" of Johne's disease to account for the reduction in incidence on vaccinated farms. He may be interested to hear that over the past few years a similar trial of the standard vaccine has been carried out in New Zealand by Dr. Buddle, and that the results obtained were similar to those in the United Kingdom, i.e. a reduction in incidence in vaccinated herds.

"Lastly, members may be interested to know that at Compton we are studying the possibility of producing non-sensitising vaccines against Johne's disease, that is, vaccines which would not interfere with the tuberculin test. No protection trials have yet been made, but vaccines which may be worthy of further study have been produced. One type of vaccine studies has been based on a methanol extract of *M. johnei*, similar to one which has been shown by Megre and by Weiss and Dubos to give protection against tuberculosis, *without* hypersensitivity. Another type under study at Compton is based on chemical treatment of Johne's bacilli. Both types of experimental vaccine are easy to produce and cause negligible local reactions. These studies are continuing."

Mr. G. N. Gould (Southampton): "As a clinician I have read the symposium on Johne's disease with great interest and it has added considerably to my knowledge of the disease. However, I am wondering just what it has taught the people in the field like myself who have the problem of trying to control it, particularly in our bull breeding and pedigree herds.

"I find it very difficult to criticise statistics, and having read the details of the limited surveys recorded I have compared these with my own impressions of the ravages of the disease. I am more interested in the losses occurring in my practice, for which I can do nothing, than in the percentage losses from Johne's disease of total cattle population or total disposals from herds. Total disposals from dairy herds do not imply that veterinary science could have done nothing to have prolonged the productive life of an animal, other than in a limited number of cases.

"When I look back to the problems of Johne's disease in my area before the war I find that it has grown enormously. The condition is undoubtedly related to changes in management and production standards, and I call Johne's disease a 'disease of habitation'. I must therefore incriminate grassland extension for its increased incidence.

"Our control of this disease must be based on early diagnosis. If we wait for chronic scour to develop then we serve no really useful diagnostic purpose. I find the complement-fixation test a little confusing from time to time when it is used as a herd test for eradication purposes. On the other hand, when it is used in herds in which clinical disease has occurred it does give one the time and the opportunity to consider the herd problem, to weigh policy within the herd and to institute control measures of husbandry which may reasonably be expected to be of some value in limiting spread.

"I find it confusing to read in one paragraph that the test as described is an unsuitable one on which to base an eradication programme, and in the next to see that it can be of limited use for eradication in dual purpose and beef herds. Used as a guide to long term control policy, the herd test—in my experience—is of great value. It gives the clinician somewhere to start in advising of all types, it does not invite the complications of wholesale slaughter of reactors, nor does it invite the need for repeated short term herd testing: it allows a constructive culling policy to be formulated.

"In regard to calf rearing, it would seem that all stock in herds where Johne's disease exists and where calves are reared, either singly or in multiple by their own or other dams, should be tested. In many of our bull breeding herds the initial herd test has a firm place in guiding calf rearing policy and the selection of calf rearers.

"Will the authors translate their thoughts to the field and tell us what they would do if they met clinical disease in a herd?"

The Replies

Dr. J. Deans Rankin, in reply to the discussion, replied first to Mr. Marr's question about whether there was any indication of the susceptibility of the Johne's bacillus to chemotherapeutic and antibiotic agents. The following antibiotic and chemotherapeutic agents had been tested for their possible effectiveness in Johne's infection: penicillin; streptomycin; chloramphenicol; Terramycin; sulphathiazole; sulphone; sulphetrone; promin; pentamidine isethionate; stilbamidine diisethionate; isonicotinic acid hydrazide; and para-aminosalycilic acid.

At a concentration of 2·0 ug/ml. streptomycin completely inhibited the growth of *M. johnei in vitro* and 2·0 to 10·0 ug/ml. isonicotinic acid hydrazide partially inhibited growth. Such levels could be obtained in the blood stream. All the other agents had either been ineffective or only acted in such high concentration as to be unworthy of further consideration.

On *in vivo* trial, however, on clinical cases of Johne's disease neither streptomycin nor isonicotinic acid hydrazide had proved effective.

The treatment of tuberculosis in man with streptomycin required the daily administration of the drug for a period of 3 months or even 6 months. This was because the drug acted on the dividing organism and the rate of division of *M. tuberculosis* was slow. *M. johnei* divided even more slowly and consequently treatment would have to be at least equally prolonged. In the few cases where such prolonged treatment had been attempted the outcome was still unsatisfactory.

Dr. Marr had also raised the question of the susceptibility of adult cattle and young animals and had asked whether there was any indication as to why the adult animal should be more resistant than the young animal. This was a difficult question to answer. It would be quite obvious to all of them that the adult animal was not just an enlargement of the young animal. This difference they knew was due to an alteration in the hormonal balance of the adult. Lurie (*loc. cit.*) in his rabbits had noticed a difference in the hormone constitution of his two strains. In the susceptible strain of rabbit he had noticed that the adrenal bodies of the animals were always heavier than in the resistant strain, and, in fact, there had been 2½ times the amount of circulating hydrocortisone in the susceptible strain. He had gone further and had exposed all of a strain of rabbits to his aerosol of human-type tubercle bacilli, then he had treated half of them with cortisone and produced in the cortisone-treated rabbits an exactly similar picture to the one he had already noted in the genetically susceptible strain. By manipulating the hormonal system of the rabbit he had produced a highly susceptible animal.

They knew that in the human being it was common for a man to break down with tuberculosis in his forties. Usually this was not a case of primary infection; he had probably been infected for a very long time and, theoretically, this slight infection should protect him—nevertheless, he broke

down. People talked of "stress", and so on, and they were coming to the view that in fact some psychological and consequent physiological upset in the man had allowed him existing infection to overcome his native resistance. It might be that a similar thing occurred in cattle. This would offer some sort of explanation for the increase in the number of clinical cases of Johne's disease when animals were moved on to new premises or when entirely new herd was being established. The animals were in some way being upset and this had a physiological effect.

Mr. Leach had asked what caused diarrhoea. Probably—and this would only apply to the badly affected animal—the lining of the bowel had become so altered that the fluid intake and the saliva swallowed was never absorbed from the bowel, but went right through it. Mr. Leach had mentioned that it appeared as if something was stimulating the animal in some way. What was definitely stimulated, of course, was the production of endothelioid cells in the intestine. It was just as if a particular cell had run wild. The animal was certainly stimulated in that respect.

Mr. Gould had asked what the veterinary surgeon was to do on a farm when presented with a Johne's disease problem. All he would say was that he had been careful to point out in his introductory remarks that he would be unable to offer any remedy at the end of the paper.

Mr. Brown had commented on the fact that they appeared to have chosen a resistant breed for the experimental animal. It was for this reason that in the paper he had dwelt at some length on the type of animal that was being used. At the time of starting these experiments some 8 or 9 years ago, there had been no factual information on breed susceptibility; it was only today, as a result of Dr. Withers' survey, that there was any real indication of a difference in susceptibility. It had taken 6 years to reach the stage where they knew that the herds supplying their experimental calves were free from *M. johnei*, and he thought that now they had to go on using these calves.

On the question of yarding animals, yards were available and so they had put the animals in them. He did not know that it made much difference.

Remarks had been made about the young animals being yarded with adults and the comparison of the infection rates in each, the inference being that the adult would not let the young animal feed, and that therefore selection was going on. In point of fact all the animals had become infected. What did happen, however, was that the young animals became clinical cases when 2 to 3 years old, but the old animals did not become clinical cases. Of 25 animals exposed in various ways as adults, only one so far had shown evidence of clinical Johne's disease. This proved that it was possible to infect an adult, but he was convinced that the adult animal when exposed to infection for the first time was much more resistant to clinical Johne's disease than the calf.

Mr. Steele-Bodger had asked whether the latent infection required a precipating factor. He thought that there was no doubt that this latent infection was present all the time in an infected herd and that something happened to individual animals which did in fact precipitate clinical disease.

Mr. H. N. Spears, in reply, dealt first with Mr. Marr's question about the number of times that vaccine had been used in the animals which had broken down in field trials. Too much attention, he said, should not be paid to the information in that table. They had that information merely because those animals had broken down; they had no similar details for the other vaccinated animals which had taken part in the trial and had remained healthy, so that comparisons were not possible.

As to how the breakdowns were distributed, of the 121 herds which had entered the trial, 39 had had breakdowns among the vaccinated stock, and about 50 per cent. of all the breakdowns had been concentrated in about 7 of those 39 herds.

Of abscess formation, he thought that Mr. Marr had been rather unlucky in his experience, but they did know that this was a problem in some herds. Why it should have been confined to certain herds they were not quite clear.

He had himself used the vaccine in 2 herds in Surrey. In one herd, which was a Guernsey herd, they had had no trouble at all on re-vaccination, but in the other one, a Shorthorn herd, they had had to abandon re-vaccination because of the gross nodules which formed. Exactly the same technique had been used in both cases.

On the subject of diagnosis of disease in vaccinated animals which became clinically suspect, they had not found faeces examination any more unreliable than it usually was, but in a small number of breakdowns with typical clinical symptoms, it had proved rather difficult to demonstrate the organisms on *post-mortem* examination.

With regard to the effect of the method of calf rearing, he had already mentioned that about 50 per cent. of all the breakdowns were concentrated in 7 herds; he knew that some of those herds had had the rather undesirable practice of buying in nursing cows and suckling calves on them, and it was not unlikely that many of the calves had been infected long before they got the vaccine. Whether that was an explanation of the breakdowns in those herds or not, he did not know, but it was a very likely one.

Dr. Chandler had asked about strains of *M. Johnnei* used in vaccine. They had been tested orally and intravenously by Doyle and had failed to set up infection. It had been very reassuring to hear Dr. Chandler's remarks about the results in New Zealand.

Mr. Bruford had mentioned disease in sheep. He did not know very much about the incidence in sheep except that it was not uncommon in the northern counties. They had issued the French type vaccine for some field trials up there.

Mr. Steele-Bodger had mentioned a Jersey herd which had a lot of Johne's disease and a copper deficiency. He was not qualified to pass an opinion on that, except to say that Marston, in Australia, when describing copper deficiency in cattle, might have been describing Johne's disease. He had no doubt that the Jersey herd in question did have Johne's disease, but it was interesting that both conditions should be so similar in symptoms. He had been interested to hear Mr. Steele-Bodger say that he had experienced no trouble on re-vaccination.

Mr. Pasfield had also brought up the subject of abscess formation on re-vaccination. In France they used the vaccine in adult animals as a diagnostic test. If the nodule failed to form or the vaccine was discharged, it was thought to indicate that the animal was infected.

Dr. F. W. Withers, in his reply, referring on Dr. Marr's remarks on breed incidence, said that he had presented the data objectively. He and Mr. Leech had been reluctant to make assertions. Mr. Leech was now working on methods by which they might get some correlation of disease incidence data with methods of husbandry, climate, management and other factors.

He was glad that Dr. Marr had raised the question of breed incidence in connection with Ayrshire and Channel Islands cows in particular, because it led to a rather interesting point. Some years ago the Agricultural Research Council Calf Survey had found a very much higher incidence of coli infection in Ayrshire calves, particularly in Scotland, than in suckled calves in the south. On some occasions one had observed that calves taken off the dam at birth did not get any colostrum at all. In the south they had found that Channel Islands calves reared on the cows were usually in very good condition. In one case in a Guernsey herd, the rearing method had been changed and calves taken off at birth on the advice of the Animal Health Division owing to the occurrence of cases of Johne's disease in the herd, and subsequently the calves were in much poorer conditions than previously when reared on the cows. So it cuts both ways—remove calves at birth to avoid Johne infection and run greater risk of white scours or *B. coli* septicaemia!

Dr. Chandler had asked about the selection of herds. Random samples had been used in all the surveys, the 11 preliminary surveys and the national survey. 15/17 preliminary samples had been drawn by the Milk Marketing Board and second stage samples by Mr. Leech. About 80 per cent. of those in the lists of herds had co-operated and produced satisfactory records. They could not know anything about the 20 per cent. who would not play.

They had no information on Johne's disease in beef herds, but in certain areas he had been told it was bad in some beef herds. Mr. Leech had raised a point about prenatal infection. Figures had been published by Chandler and by Cunningham & Gilmour giving 0·45 and 0·63 per cent. for what might be regarded as prenatal or very early infection.

These were very close to the incidence of clinical cases in adult stock, and also to that for diagnosed and suspected cases in vaccinated stock in field trials. This suggested the possibility that it might be the animal infected prenatally or very early in life which was going to be a clinical case in any event, whether vaccinated or not. However, these figures might be pure coincidence.

Mr. Gould had referred to total disposals. The speaker thought that 99 per cent. of the animals recorded as clinical cases were disposed of pretty quickly. Years ago Mr. Doyle had warned him about cases disposed of by the experienced stockman who was able to spot the very early case and get rid of it before any clinical symptoms appeared. He thought the incidence would be increased if such cases were known. There was also probably something in the point about Channel Islands cattle being kept longer; perhaps more of them therefore became clinical cases, whereas the salvage value of other breeds favoured early disposal.

Mr. Steele-Bodger had mentioned the relationship between tuberculosis and Johne's disease. Mr. Peck's knackery survey in Devonshire had shown that many animals died or were disposed of *in extremis* suffering from more than one disease. In many cases two or three diseases were present.

The samples worked on and the analysis of the material had been done by Mr. Leech and his staff at Rothamsted and he paid a tribute to that work. Mr. Leech should be, if not in his place, at least on the platform with him.

Mrs. M. H. Maclay, in her reply, referring to Dr. Marr's question about the proportion of reactions in herds free from clinical disease that were of a temporary nature, said that they had very little evidence on this point, because initially their herd tests had been made on herds with clinical disease and in most cases where repeated testing had been carried out reactors had been eliminated. What little evidence they had collected on infected herds suggested that temporary reactions were most frequent in animals about two years old and that such an initial reaction after one or more negative tests might be followed in those herds by a more permanent reaction and ultimate evidence of infection.

The mere fact that the reactions fluctuated could not always be taken as evidence of recovery, because they also got the occasional reversion to a negative reaction in an animal which subsequently proved positive on *post-mortem* examination.

With regard to the more extensive use of herd testing at the public expense, this was more a question of whether the benefits obtained were considered to justify the expenditure involved. It was undoubtedly a question to which the Ministry would give serious consideration. But the permanent marking of reactors was something that would require considerable thought in view of the possibility of a temporary or non-specific reactions. Even in tuberculin testing the reactors had not been dealt with in this manner.

Mr. Brown had asked about the long period between infection and the production of an antibody in comparison with the reaction after vaccination. She thought that the explanation of that might be that the injection of a large number of organisms was a much stronger antigenic stimulus than occurred in natural exposure to infection with Johne's disease, which was extremely insidious and a slowly progressive infection.

As to the question about familial susceptibility and prenatal infection, they did not consider that the available evidence suggested that prenatal infection occurred in non-clinical animals. In the chart giving the results of P.M. examination of non-clinical blood reactors 11 of the 20 confirmed cases were carrying calves (8 near full term and 3 earlier in gestation). Microscopical and cultural examination of these foetuses failed to disclose any infected; and she did not think that anybody else had yet reported prenatal infection in non-clinical cases.

In the "family tree", in a number of instances one and sometimes two generations were missed, so that it was not a direct link in every case.

The action to be taken over an animal giving a weak positive reaction, following a good lactation and no clinical signs of any sort, depended upon the policy being followed. If the blood test was being used for repeated testing with the object of eradication, such an animal should be slaughtered, but if it were permissible to use the test simply as a means of control, in a less rigorous fashion, it was possible that there would be grounds for retaining a weak positive and re-testing it later if it were showing no other signs, and particularly if it were a young animal.

Mr. Steele-Bodger had asked about herd testing and availability. The herd testing so far done had been experimental work. They obviously could not take on unlimited experimental work.

In reply to Mr. Bruford, at Weybridge they had only a limited amount of information about sheep. They knew that there was quite a bit of Johne's disease in sheep in Cumberland, and no doubt in other parts of the country also. They had now, in conjunction with the Penrith Centre, carried out vaccination experiments, but there were no results yet. Blood test was of very limited value in sheep, which was possibly due to the difference in strain of organism.

With regard to Mr. Gould's point, she could not at present give any constructive answer.

The Chairman, in thanking the authors of the papers and the opener of the discussion, said that the contributors must indeed be gratified that there had been such a large attendance at the session and that so many members had stayed to the end of this last scientific session of the Congress.

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